EFFECT OF TEMPERATURE AND LIGHT ON THE PHOTOSYNTHESIS AS MEASURED BY CHLOROPHYLL FLUORESCENCE OF CULTURED *Kappaphycus* sp. (*SAKOL* STRAIN) FROM INDONESIA

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ABSTRACT

Photosynthetic performance of *carrageenophyte* (Solieriaceae; Rhodophyta) cultured in Indonesia, *Kappaphycus* sp. (*Sakol* strain), was investigated at various temperature and light conditions related to their cultivation performance. A "pulse-amplitude modulated-chlorophyll fluorometer" (Diving-PAM) was used to generate a rapid light curves (RLCs) to provide estimates relative electron transport rates (rETR) for over 10 temperatures (i.e., 16°C to 34°C) and at nine photosynthetic active radiation (PAR) levels, which ranged from 0 to 1,000 mol photons m^{-2} s^{-1}. The initial slope (α), photo-inhibition coefficient (β), and the coefficient of maximum photosynthesis assuming no photo-inhibition (γ) is calculated by fitting the RLCs on a nonlinear model by using a two-level hierarchical Bayesian model. The experimental results showed that *Kappaphycus* sp. required temperatures ranging from 26°C to 34°C to maintain their high levels of photosynthetic activity. Saturating irradiace (E_k) at the temperature range occured ranging from 120 to 150 µmol photons m^{-2} s^{-1}. The model equation that have been derived from this series experiment can be used to determine the requirement of temperature and light intensity (irradiance) of any seaweed species.

KEYWORDS: **chlorophyll fluorescence, irradiance, *Kappaphycus* sp. (*Sakol* strain), temperature**

Introduction

Cultivation of red algae (Rhodophyta) genera *Eucheuma* and *Kappaphycus* has supplied a large amount of *carrageenan* in the world (Dawes, 1994). *K. alvarezii* is largely cultivated in tropical regions especially in Indonesia and the Philippines. In 2009, total world production was 160,000 dry tons where 146,000 tons of dry weight produced from these two countries (Indonesia and the Philippines). In details, 61,000 dry tons is produced in the Philippines and 85,000 dry tons is produced in Indonesia (Bixler & Porse, 2011). Commercial cultivation of *Kappaphycus* was developed in the Philippines during the latter half of the 1960's using local varieties selected from the wild (Parker, 1974). It has also been reported that cultivation of *Kappaphycus* has been successful in China (Wu *et al.*, 1989), Madagascar (Mollion & Braud, 1993), and Vietnam (Ohno *et al.*, 1996).

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The relative success of seaweed cultivation is apparently due to similar environmental conditions between the recipient transplanted sites and the site where the *Eucheuma* was originally collected (Ohno *et al.*, 1994). Temperature and light are the main environmental factors that affect the process of photosynthesis on *Euchemua* and *Kappaphycus* (convert light into chemical energy molecules such as sugars and organic matter). Therefore, detailed information related to how water temperature and light affecting the photosynthetic process is needed to be determined in order to optimize the cultivation system.

Several strains of *Kappaphycus alvarezii* are cultured in Indonesia such as *Sakol*, *Tambalang*, *cottonii*, and *Sumba*. *Sumba* strain (*Kappaphycus* sp.) is predicted coming from Sumba Island in Indonesia, while *Sakol* and *Tambalang* strain are originally from the Philippines. Although, *Sakol* strain is prominent commodity cultured in Indonesia, study on their physiological aspects in particular photosynthesis is very limited. Species of *K. alvarezii* is mostly used for experiment (Dawes *et al.*, 1994; Ohno *et al.*, 1994; Wobeser *et al.*, 2001), and study on photosynthesis of *K. alvarezii* was conducted by measuring dissolved oxygen evolution and with irradiance less than 650 μmol photons m^{-2} s^{-1} (Wobeser, 2001; Lideman *et al.*, 2011). Studies of *Eucheuma* and its related genera have been reported from a variety of locations in the world since the 1970's (*e.g.*, Dawes *et al.*, 1974; Paula *et al.*, 2001; 2002; Hung *et al.*, 2009), and they have contributed towards the elucidation of their characteristics of growth and *carrageenan* contents to conserve natural resources and enhanced the cultivation techniques and supply.

Since the late 1980s, pulse amplitude modulated (PAM)-chlorophyll fluorometry has been performed for seagrasses as well as many terrestrial plants (Beer *et al.*, 1998; Beer & Björk, 2000; Aldea *et al.*, 2006; Kuster *et al.*, 2007; Ralph *et al.*, 1998, 2006) as a quick and efficient way of evaluating the photosynthetic response of whole intact plants. PAM has also been used in aquatic research for macroalgae to elucidate photosynthetic response (Renger & Schreiber, 1986; Huppertz *et al.*, 1990; Gevaert *et al.*, 2002; Enriquez & Borowitzka, 2011). PAM can be an efficient way of clarifying the response of cultured plants of *Eucheuma/Kappaphycus* (Aguirre-von-Wobeser *et al.*, 2001) and a battery of tests could be designed to clarify the optimal temperature and irradiance conditions that can maximize photosynthesis (i.e., growth) and also be used to diagnose the physiological condition of cultivated macroalgae such as health condition.

This present study focused on elucidating the temperature and irradiance conditions needed to promote photosynthesis of cultured *Kappaphycus* sp. (*Sakol* strain) from Indonesia using PAM fluorometry.

MATERIALS AND METHODS

Specimen Collection and Stock Maintenance

Cultured specimens of *Kappaphycus* sp. (*Sakol* strain) (Figure 1) were collected in culture area in Funaga, Takalar, South Sulawesi, Indonesia (5°34'56.62"N, 119°27'42.56"E).

Ten bunches of *Sakol* strain which were cultured at the depth of 1-2 m were collected into 1,000-mL plastic bags and stored in a cool box at approximately 24°C. The specimens were directly transported to Japan within 2 days through Kagoshima Maru, a research vessel of Fisheries Faculty of Kagoshima University which was anchored in Benoa Port, Bali, Indonesia. In the laboratory, specimens were maintained in a tank (1.0 m x 1.0 m x 0.5 m) containing seawater with salinity around 33 ppt and pH around 8.0. Water temperature was maintained at 24°C using water temperature controller. Photosynthetic active radiation (PAR) was provided by room fluorescent lamps at approximately 90 μmol photons m^{-2} s^{-1} with 12:12 light/dark cycle. The samples were acclimatized for around 4-h before experiment. Voucher herbarium specimens were deposited in the herbarium of Marine Botany, Kagoshima University Museum (KAG).

Figure 1. Specimen of cultured *Kappaphycus* sp. (*Sakol* strain)

Rapid Light Curves

Rapid light curves (RLCs) were generated by running the standard algorithm of the PAM fluorometer (Diving-PAM, Heinz Walz GmbH, Germany) using an incremental sequence of actinic illumination periods with PAR intensities increasing in nine steps from 0 to 1,000 μmol photons m^{-2} s^{-1}. Relative electron transport rate (rETR) was calculated using the equation as indicated below:

$$rETR = 0.5 . Y. PAR . AF \quad (1)$$

where: *Y* is the effective quantum yield of PSII ($\Phi_{PSII} = (F - F_m')/F_m'$, where *F* is the initial fluorescence, and F_m' is the maximum fluorescence), the factor 0.5 assumes that half of the photons are absorbed by PSII (Schreiber *et al.*, 1995), and AF is the fraction of incident light assumed to be absorbed by the sample (i.e., 0.84).

Temperature and Light Effect on Photosynthetic Parameters

From each collected specimen, 2-cm long portions of the thalli were placed in a 50 cm x 40 cm x 50 cm plastic tank with four replicates. Temperature was adjusted and maintained by temperature controlled water bath. Temperature in the tank was measured with a thermocouple to confirm that the water reached the desired temperature. The relative electron transport rates were determined by generating RLCs with nine PAR levels in every 2°C interval ranging from 16°C to 34°C. When the temperature changed, we spent for at least 1 h to change 2°C. We also acclimatized the algae for more than 1 h before each temperature experiment. We modeled the rETR versus PAR to calculate the maximum rETR rate in the absence of photo-inhibition (γ), the initial slope (α) of the photosynthesis-irradiance curve (*P-I* curve), and the photo-inhibition coefficient (β) by fitting the RLCs to a non-linear model modified after Platt *et al.* (1980):

$$rETR = \gamma \cdot \left(1 - \exp\left(-\frac{\alpha}{\gamma} \cdot PAR\right)\right) \cdot \left(\exp\left(-\frac{\beta}{\gamma} \cdot PAR\right)\right) \quad (2)$$

Based on these parameters, the values of E_{opt} is estimated, which defines PAR when the rETR is at a maximum ($rETR_{max}$) and E_k which defines PAR when rETR begins to saturate.

$$\frac{drETR}{dPAR} = \alpha \exp\left(-\frac{\beta}{\gamma} PAR - \frac{\alpha}{\gamma} PAR\right) - \beta\left(1 - \exp\left(-\frac{\alpha}{\gamma} PAR\right)\right) \exp\left(-\frac{\beta}{\gamma} PAR\right) \quad (3)$$

Furthermore, by computing the derivative of Eq. 3 with respect to PAR, and solving the equation when $\frac{drETR}{dPAR} = 0$, the value of PAR at the rETR maxima (E_{opt}) can be estimated from the first real root:

$$E_{opt} = \frac{\gamma}{\alpha} \ln\left(\frac{\alpha}{\beta} + 1\right) \quad (4)$$

by substituting E_{opt} into Eq. 2, we arrive at the value of rETR at the maxima ($rETR_{max}$) of the *P-I* curve. Saturating PAR (E_k) was calculated using the equation:

$$E_k = \frac{rETR_{max}}{\alpha} \quad (5)$$

Statistical Analysis

Statistical analysis of photosynthetic parameters of γ, α, and β were done using *R* (R Development Core Team, 2011), and model fitting was performed using OpenBUGS (Thomas *et al.*, 2006). The parameters were examined by fitting the RLCs to the non-linear model (Eq. 2) using hierarchical Bayesian methods. OpenBUGS primarily uses a Gibbs sampler to construct the posterior distributions of the parameters, and 4 chains of at least 20,000 samples were generated and assessed for convergence. Uniform priors were placed on all of the hyper parameters of the model.

Model formulation and selection of the relationship between the estimated parameters and experimental water temperature were also examined based on the methods used by Bhujel (2008). Based on the model results, a range of optimal temperatures for the photosynthetic activity of these species could be defined as at least 95% of the estimated maximum or minimum parameter values. A general linear model was used to examine temperature and species effect and the interaction of them on the photosynthetic parameters. Levene's homogeneity test was used to test of equality of error of variances. The curve estimation and general linear model were analyzed by using SPSS v.17 (SPSS Inc.).

RESULTS AND DISCUSSION

Results

Rapid Light Curves (RLCs)

Generally the rapid light curves of this species increased until reaching some a symptote as most often observed in photosynthesis-irra-

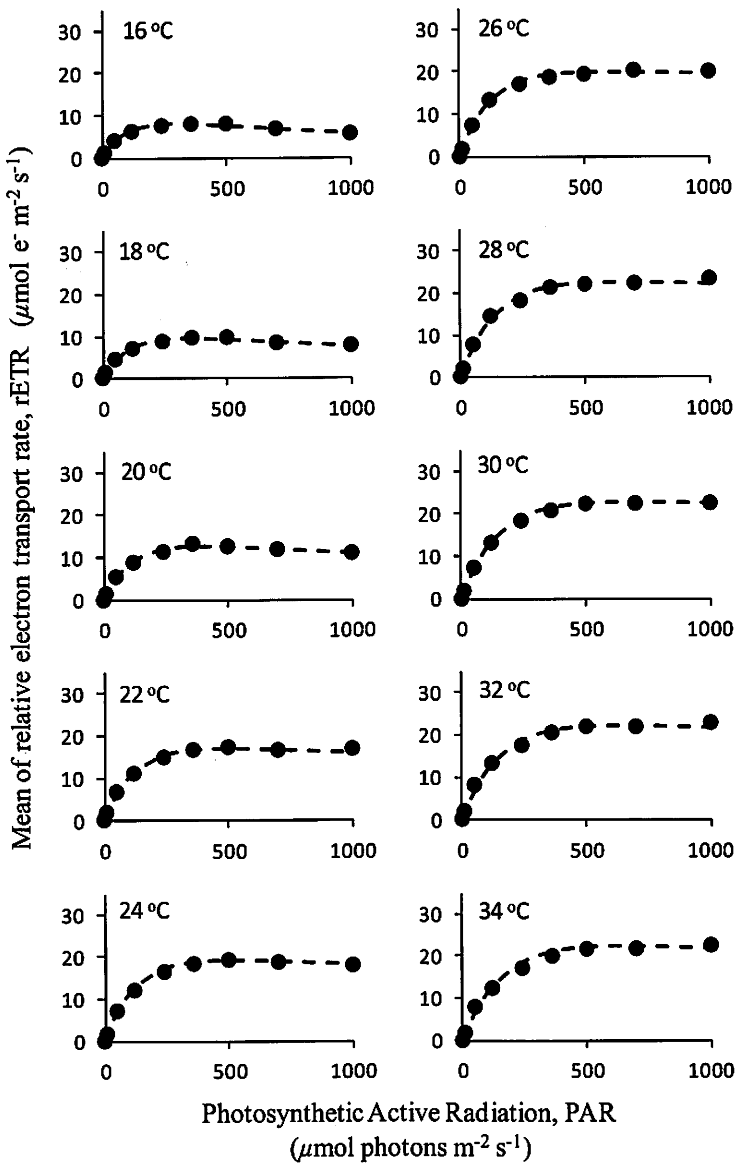

Figure 2. The rapid light curves of rETR (dot) of *Kappaphycus* sp. determined over a temperature gradient of 16°C-34°C. The dash lines indicate the fitted model of *Kappaphycus* sp. n = 4 samples for each temperature and PAR

diance curves (*P-I* curve), and photo-inhibition was not readily apparent until PAR reached 1,000 µmol m^{-2} s^{-1} (Figure 2). By fitting Eq. 4 to the results using hierarchical Bayesian methods, it was able to elucidate the parameters of the model (γ, α, and β) across all water temperatures, as well as derive estimates of $rETR_{max}$, E_k, and E_{opt}.

Effect of Temperature on the Photosynthetic Parameters

The mean value of maximum rETR in the absence photo-inhibition (γ) ranged from 10.1-24.3 µmol e^- m^{-2} s^{-1} over of temperature examined and they monotonically increased with increasing temperature (Figure 3a). The parameter γ for this *Kappaphycus* sp. increased linearly with increasing of temperature ($F_{(1,39)}$ = 142,344; P<0.001). The models of γ vs temperature (*t*) could be described by equation: $\gamma = -1.9414 + 0.8448\ t$ ($R^2 = 0.766$).

The mean values of the initial slope (α) of *Kappaphycus* sp. ranged from 0.0996-0.1738 µmol e^- (µmol photons)$^{-1}$ and were dome-shaped with respect to temperature (Figure 3b). The model between temperature and α can be described by a quadratic equation ($F_{(2,39)}$ = 56,575; P<0.001) and they could be described by quadratic equation: $\alpha = -0.2569 + 0.0306\ t - 0.0005\ t^2$ ($R^2 = 0.754$). Based on these model, the highest α of *Kappaphycus* sp. was estimated at 0.2112 µmol e^- (µmol photons)$^{-1}$ and occured at temperature of 32.1°C. Hence, 95% of maximum α value was 0.2006 µmol e^- m^{-2} s^{-1} which lead to temperature range of 26.6°C-34.0°C.

The mean values of the photo-inhibition coefficient (β) of these species ranged from 0.0012 to 0.0056 µmol e^- (µmol photons)$^{-1}$ and were U-shaped in nature (Figure 3c). This parameter can also be modeled as a quadratic relationship with temperature ($F_{(2,39)}$ = 25.610, P<0.001) and they could be described by equation: $\beta = 0.0228 - 0.0015\ t + 0.0000266\ t^2$ ($R^2 = 0.581$). A β minimum could be estimated at temperatures near 0.0165 µmol e^- m^{-2} s^{-1} at temperature of 28.2°C. In case of β, 95% of the minimum value would be 0.00174 µmol e^- m^{-2} s^{-1} which leads to a temperature range of 26.4°C-30.0°C.

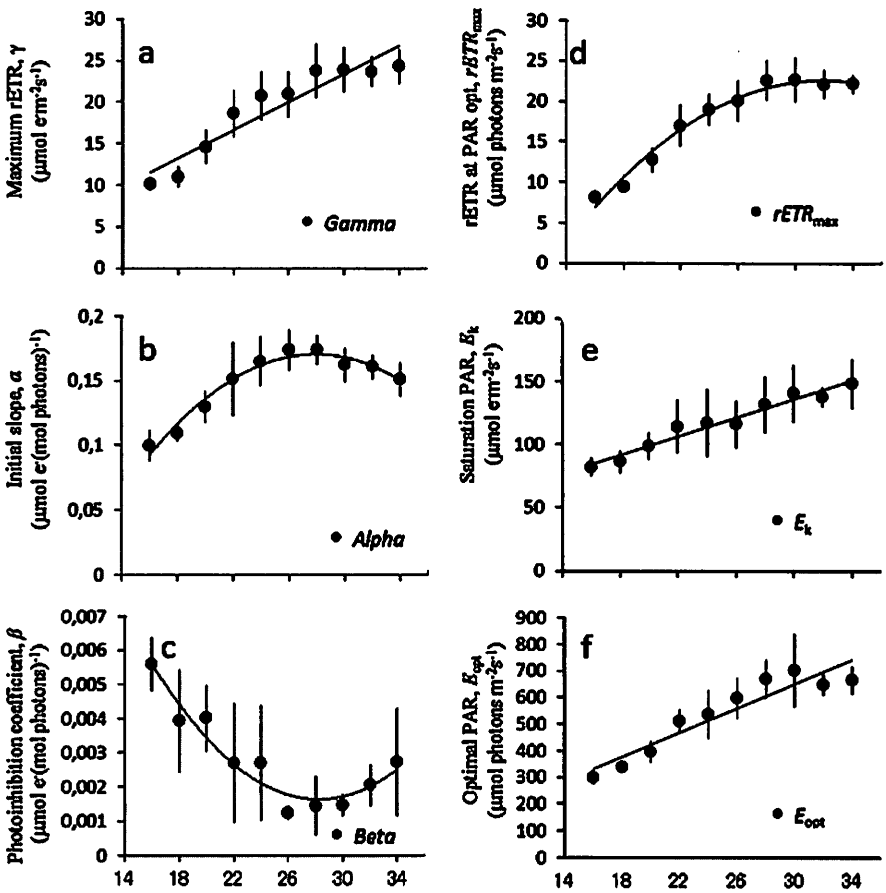

Figure 3. Photosynthetic parameters (dot) and fitted model (line) at some temperature levels of cultured *Kappaphycus* sp. (*Sakol* strain). Bars indicate a standard deviation. n = 4 samples for each temperature and PAR

The mean values of $rETR_{max}$ of *Kappaphycus* sp. ranged from 8,129-22,683 μmol $e^- m^{-2} s^{-1}$ (Figure 3d). A model describing the relationship between temperature (*t*) and rETRmax of *Kappaphycus* sp. was quadratic in form ($F_{(2.39)}$ = 32,807; P<0.001). The relationship between temperature and rETRmax could be described by equation: $rETR_{max}$ = - 40.62 + 3.9611 t - 0.062 t^2 (R^2 = 0.639). Based on the model, maximum value of rETRmax was 22.6 μmol $e^- m^{-2} s^{-1}$ and occured at temperature of 31.9°C. Moreover, for $rETR_{max}$, 95% of the maximum would be 22.5 μmol $e^- m^{-2} s^{-1}$, which leads to a temperature range of 27.6°C–34.0°C.

Regarding the mean values for E_k, which indicates the value of PAR when rETR began to saturate, they increased by increasing temperature (Figure 3e). A model describing the relationship between temperature and E_k of *Kappaphycus* sp. was linear ($F_{(2.39)}$ = 65,831; P<0.001). Furthermore, the relationship between temperature and E_k of *Kappaphycus* sp. could be described by the equation: E_k = 24.943 + 3.6895 t (R^2 = 0.632). Their E_k values are shown in Table 1.

The PAR, where $rETR_{max}$ was observed (at E_{opt}), increased with increasing temperature (Figure 3f). A model describing the relationship between temperature and E_{opt} of *Kappaphycus* sp. was linear in form ($F_{(2.39)}$ = 114,079; P<0.001) and could be described by equation: E_{opt} = 32.313 + 22.777 t (R^2 = 0.750). Their E_{opt} values are shown in Table 1.

Table 1. Estimated saturating PAR (E_k) and maximum PAR (E_{opt}) of *Kappaphycus* sp. based on curve model estimation

Temperature (°C)	E_k	E_{opt}
16	84.0	1,945.0
18	91.4	2,113.1
20	98.7	2,281,2
22	106.1	2,449.2
24	113.5	2,617.3
26	120.9	2,785.4
28	128.2	2,953.4
30	135.6	3,121.5
32	143.0	3,289.6
34	150.4	3,457.7

E_k = 24.943 + 3.6895 9 (R^2 = 0.632)

E_{opt} = 32.313 + 22.777 t (R^2 = 0.750)

DISCUSSION

Rapid Light Curve

Generally the rapid light curves (RLCs) of the species increased until reaching an asymptot, and photo-inhibition was not readily apparent until PAR reached 1,000 μmol $m^{-2} s^{-1}$ (Figure 2) and its RLCs shape were similar to *Kappaphycus* sp. (*Sumba* strain) and *Eucheuma denticulatum* as reported in our previuos study (Lideman *et al.*, 2013). The similarity of the RLCs shape of these two species probably because both grow in tropical region, Indonesia. If compared to *Meristotheca papulosa* and *M. coacta*which are grown in sub-tropical area in southern Japan, their RLCs shape were hump-shape and expressed clear photo-inhibition at higher temperature as found in our previous experiment (Lideman *et al.*, 2012). These findings indicated that temperature of habitat affected the photosinthetic activity of seaweed species.

Effect of Temperature on Photosintetic Parameter

Based on the value of α and β which both are the coefficient of photosynthesis and photo-inhibition, respectively, this species seemed sensitive to light change. In addition, both coefficients showed dependence on temperature (Figure 3 band 3c), which significantly affected the ability of photosynthetic of the species. The finding clearly showed that these coefficients reached a maximum value (i.e., α) or minimum value (i.e., β) within similar temperature ranges which highly correlated to the environmental temperatures ranged from 25°C-30°C (Thomascik *et al.*, 1997).

$rETR_{max}$ parameters gave an overview of the productivity of seaweed to varying degrees temperature (Figure 3d). At temperature ranged from 27.6°C–34.0°C, $rETR_{max}$ showed the maximal range. The temperature range is actually correlated with the water temperature recorded in the farming area (Sugiarto *et al.*, 1976; Nonji, 1993; Tomascik *et al.*, 1997; Amin, 2008). Furthermore, the temperature range for the maximum $rETR_{max}$ was also closed to estimation based on a study on "dissolved oxygen evolution" where maximum photosynthesis (P_{max}) and respiration of *Kappaphycus alvarezii* (morfotypered and green coming from the Philippines) reached maximal values at 30°C (Wobeser *et al.*, 2001). Other studies on photosynthesis and growth showed that *K. alvarezii* has high grow that temperature of 25°C-28°C (Ohno *et al.*, 1994) and optimal growth of *Meristotheca papulosa* is between 20°C-24°C (Lideman *et al.*, 2011).

The range of parameters described above are individually and less suitable to interpretate a good range. Combined three parameters of α, β, and $rETR_{max}$, should be used to determine the optimal temperature range describing the photosintetic activity for the cultivation of this seaweed. Based on combined three parameters (α, β, and $rETR_{max}$), it was found that optimum temperature for the photosynthetic activity of the *Sakol* strain ranged from 26°C-34°C. Furthermore at the optimal temperature range, photosynthetic rate reached the saturation level (E_k) at irradiance of 120-150 μmol photons $m^{-2}s^{-1}$ and E_{opt} was between 1,945-3,457 μmol photons $m^{-2}s^{-1}$. This optimal temperature range is relatively similar with others tropical carrageenophyte, for example, *Eucheuma denticulatum* which was 23°C-32°C and *Kappaphycus* sp. (*Sumba* strain) was 22°C-33°C (Lideman *et al.*, 2013).

The saturating irradiance (E_k) value of the *Sakol* strain (120-150 μmol photons m^{-2} s^{-1}) investigated in this experiment was relatively similar with E_k value of *E. denticulatum* (119.7-151.7 μmol photons m^{-2} s^{-1}) and *Kappaphycus* sp. (*Sumba* strain; 122.5-167.1 μmol photons m^{-2} s^{-1}), while the E_{opt} value at the optimum temperature range was higher than *E. denticulatum* (531.2-616.9 μmol photons m^{-2} s^{-1}) and *Kappaphycus* sp. (*Sumba* strain) with value of 585.5-648.8 μmol photons m^{-2} s^{-1} (Lideman *et al.*, 2013). This higher E_{opt} value indicated that this species may grow at higher light intensity as a typical of tropical species and also described why this species showed a low photo-inhibition value at higher light intensity (Figure 2). Measured irradiance of this species in farming area at noon time was between 109-1,482 μmol photons m^{-2} s^{-1} in water deepth of 0.5-2 m (Lideman *et al.*, 2013), and E_k value showed in the range of measured irradiance.

CONCLUSION

Kappaphycus sp. required temperatures ranging from 26°C to 34°C to maintain their high levels of photosynthetic activity. Saturating irradiace (E_k) at the temperature range was occured ranging from 120 to 150 μmol photons m^{-2} s^{-1}. Model equations that have been derived from this series experiment can be used to determine the requirement of temperature and light intensity (irradiance) of any seaweed species. However, further field study is necessary to be carried-out to apply models found in this study.

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REFERENCES

Aguirre-von-Wobeser, E., Figueroa, F.L., & Cabello-Pasini, A. 2001. Photosynthesis and growth of red and green morphotypes of *Kappaphycus alvarezii* (Rhodophyta) from the Philippines. *Mar. Biol.*, 138: 679-686.

Aldea, M., Frank, T.D., & DeLucia, E.H. 2006. A method for quantitative analysis of spatially variable physiological processes across leaf surface. *Photosynth. Res.*, 90: 161-172.

Amin, M., Rumayar, T.P., Femmi, N.F., Keemur, D., & Suwitra, I.K. 2008. The assessment of seaweed (*Eucheuma cotonii*) growing practice of different systems and planting seasons in Pangkep Regency, Central Sulawesi. *Indonesian Journal of Agriculture*, 1(2): 132-139.

Beer, S., Vilenkin, B., Weill, A., Veste, M., Susell, L., & Eshel, A. 1998. Measuring photosynthetic rates in seagrasses by pulse amplitude modulated (PAM) fluorometry. *Mar. Ecol. Prog. Ser.*, 174: 293-300.

Beer, S. & Björk, M. 2000. Measuring rates of photosynthesis of two tropical seagrasses by pulse amplitude modulated (PAM) fluorometry. *Aquatic Bot.*, 66: 69-76.

Bhujel, R.C. 2008. Statistics for aquaculture. Wiley-Blackwell, Iowa, USA.

Bixler, H.J. & Porse, H. 2011. A decade of change in seaweed hydrocolloids industry. *J. Appl. Phycol.*, 23: 321-355. doi: 10.1007/s10811-010-9529-3

Dawes, C.J., Mathieson, A.C., & Cheney, D.P. 1974. Ecological studies of Floridian

Eucheuma (Rhodophyta, Gigartinales). I. Seasonal growth and reproduction. *Bull. Mar. Sci.*, 24: 235-273.

Dawes, C.J., Lluisma, A.O., & Trono, G.C. 1994. Laboratory and field growth studies of commercial strains of *Eucheuma denticulatum* and *Kappaphycus alvarezii* in the Philiphines. *J. of Appl. Phycol.*, 6: 21-24.

Enriquez, S. & Borowitzka, M.A. 2011. The use of the fluorescence signal in studies of seagrass and macroalgae. *In* Suggett, D.J., Prášil, O., & Borowitzka, M.A. (Eds.), Chlorophyll a fluorescence in aquatic sciences, methods and developments. p. 187-208. Developments in Applied Phycology 4, Springer, Dordrecht.

Gevaert, F., Creach, A., Davoult, D., Holl, A.C., Seuront, L., & Lemoine, Y. 2002. Photoinhibition and seasonal photosynthetic performance of the seaweed *Laminaria saccharina* during a simulated tidal cycle: chlorophyll fluoromety measurements and pigment analysis. *Plant, Cell and Environment*, 25: 859–872.

Hung, L.D., Hori, K., Nang, H.Q., Kha, T., & Hoa, L.T. 2009. Seasonal changes in growth rate, carrageenan yield, and lectin content in the red alga *Kappaphycus alvarezii* cultivated in Camranh Bay, Vietnam. *J. of Appl. Phycol.*, 21: 265-272.

Huppertz, K., Hanelt, D., & Nultsch, W. 1990. Photoinhibition of photosynthesis in the marine brown algae *Fucus serratus* as studied in field experiments. *Marine Ecology Progress*, 66: 175-182.

Kuster, A., Pohl, K., & Altenburger, R. 2007. A Fluorescence-based bioassay for aquatic macrophytes and its suitability for effect analysis of non-photosystem II inhibitors. *Env. Sci. Pollut. Res.*, 14: 377-383.

Lideman, Nishihara, G.N., Noro, T., & Terada, R. 2011. *In vitro* growth and fotosynthesis of three edible seaweeds, *Betaphycus gelatinus*, *Eucheuma serra* and *Meristotheca papulosa* (Solieriaceae, Rhodophyta). *Aquaculture Sci.*, 59(4): 563-571.

Lideman, Nishihara, G.N., Noro, T., & Terada, R. 2012. Effect of temperature and light on the photosynthetic performance of two edible seaweeds: *Meristotheca coacta* Okamura and *Meristotheca papulosa* J. Agardh (Solieriaceae, Rhodophyta). *Aquaculture Sci.*, 60(3): 377-388.

Lideman, Nishihara, G.N., Noro, T., & Terada, R. 2013. Effect of Temperature and Light on the photosynthesis as measured by chlorophyll fluorescence of cultured *Eucheuma denticulatum* and *Kappapycus* sp. (*Sumba* strain) from Indonesia. *J. of Appl. Phycol.*, 25(2): 399-406. DOI.10.1007/s10811-012-9874-5

Mollion, J. & Braud, J.P. 1993. A *Eucheuma* (Solieriacea, Rhodophyta) cultivation test on the south-west coast of Madagaskar. *Hydrobiologia*, 260/261: 373-378.

Nonji, A. 1993. Indonesian Ocean. Djambatan. Jakarta.

Ohno, M., Largo, D.B., & Ikumoto, T. 1994. Growth rate, carrageenan yield and gel properties of cultured kappa-carrageenan producing red alga *Kappaphycus alvarezii* (Doty) Doty in the subtropical waters of Shikoku, Japan. *J. of Appl. Phycol.*, 6: 1-5.

Ohno, M., Nang, H.Q., & Hirase, S. 1996. Cultivation and carrageenan yield and quality of *Kappaphycus alvarezii* in the waters of Vietnam. *J. Appl. Phycol.*, 8: 431-437.

Parker, H.S. 1974. The culture of the red algal genus *Eucheuma* in the Filipina. *Aquaculture*, 3: 425-439.

Paula, E.J., Erbert, C., & Pereira, R.T.L. 2001. Growth rate of the carrageenophyte *Kappaphycus alvarezii* (Rhodophyta, Gigartinales) in vitro. *Phycol. Res*,. 49: 155-161.

Paula, E.J., Pereira, R.T.L., & Ohno, M. 2002. Growth rate of the carrageenophyte *Kappaphycus alvarezii* (Rhodophyta, Gigartinales) introduced in subtropical waters of São Paulo State, Brazil. *Phycol. Res.*, 50: 1-9.

Plat, T., Gallegos, C.L., & Harrison, W.G. 1980. Photoinhibition of fotosintesis in natural assemblages of marine phytoplankton. *J. Mar. Res.*, 38: 687-701.

R Development Core Team. 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org

Ralph, P.J., Gademann, R., & Dennison, W.C. 1998. In situ seagrass fotosintesis measured using a submersible, pulse-amplitude modulated fluorometer. *Marine Biology*, 32: 367-373.

Ralph, P.J., Schreiber, U., Gademann, R., Kuhl, M., & Larkum, A.W.D. 2006. Coral photobiology studied with a new imaging pulse amplitude modulated fluorometer. *J. Phycol.*, 41: 335-342.

Renger, G. & Schreiber, U. 1986. Practical applications of fluorometric methods to algae and higher plant research. *In* Light emission by plants and bacteria. Edited by Govindjee, J. & Amesz, D.D. (Eds.) Fork Academic Press, Orlando.

Schreiber, U., Hormann, H., Neubauer, C., & Klughammer, C. 1995. Assessment of photosystem II, photochemical quantum yield by chlorophyll fluorometу quenching analysis. *Aust. J. Plant Physiol.*, 22: 209-220.

Soegiarto, A., Birowo, S., & Sukarno. 1976. Map of Indonesian Ocean. Indonesian Science Institute.

Thomas, A., O'Hara, B., Ligges, U., & Sturtz, S. 2006. Making BUGS Open. *R News*, 6: 12-17.

Tomascik, T., Mah, A.J., Nonji, A., & Moosa, M.K. 1997. The ecology of Indonesian Seas. Periplus Editions (HK) Ltd. Sidney, Jakarta, Kanagawa, Oxford, Singapore, North Clarendon.

Wobeser, E.A., Figueroa, F.L., & Cabello-Pasini, A. 2001. Fotosynthesis and growth of red and green morphotypes of *Kappaphycus alvarezii* (Rhoddophyta) from the Philipines. *Marine Biology*, 138: 679-686.

Wu, C., Li, J., Xia, E., Peng, Z., Tan, S., Li, J., Wen, Z., Huang, X., Cai, Z., & Chen, G. 1989. On the transplantation and cultivation of *Kappaphycus alvarezii* in China. *J. Oceanol. Limnol.*, 7: 327-334.