

**Effect of Temperature and Light on the Photosynthetic
Performance of Two Edible Seaweeds: *Meristotheca coacta*
Okamura and *Meristotheca papulosa*
J. Agardh (Solieriaceae, Rhodophyta)**

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Abstract: The photosynthetic performance of two species of *Meristotheca* (Solieriaceae, Rhodophyta), *M. coacta* and *M. papulosa*, was investigated under a variety of temperature and light conditions to derive basic information regarding their physiology. A pulse amplitude modulated-chlorophyll fluorometer (Imaging-PAM) was used to generate rapid light curves (RLCs) to provide the relative electron transport rates (rETR) over 21 levels of photosynthetic active radiation (PAR), ranging from 0 to 1,078 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ at 14 temperatures (i.e., from 8 to 34°C). The initial slope (α), photoinhibition (β) and coefficient (γ) was calculated by fitting the RLCs to a nonlinear model of the form $rETR = \gamma (1 - \exp(-\alpha \cdot PAR/\gamma)) (\exp(-\beta \cdot PAR/\gamma))$ using a two-level hierarchical Bayesian model. Both species required temperatures ranging from 18 to 28°C to maintain optimal photosynthetic activity, as revealed by the estimated model parameters. The optimal PAR (PAR_{opt}) increased with increasing temperature. *Meristotheca coacta* and *M. papulosa* can be considered well-adapted to the current natural light and temperature conditions of southern Kyushu, Japan. Finding in this study should be useful to the design and manage mariculture programs to conserve the natural resources.

Key words: *Meristotheca coacta*; *Meristotheca papulosa*; Photosynthesis; Temperature

The genus *Meristotheca* (Solieriaceae, Rhodophyta) is known to be widely distributed in the Indo-Pacific area, and can often be found along the shores of southern Japan (Yoshida 1998; Faye et al. 2005, 2007). In Japan, three species of *Meristotheca*, *M. coacta* Okamura (Fig. 1a), *M. imbricata* Faye et Masuda and *M. papulosa* (Montagne) J. Agardh (Fig. 1b), can be observed (Yoshida and Yoshinaga 2010), and which *M. imbricata* is a newly described endemic species to this region (Faye et al. 2008).

Meristotheca papulosa is one of the popular edible seaweeds and is used as the ingredient

for salads in Japan, especially in the prefectures of Kochi, Kumamoto, Miyazaki and Kagoshima (Ohno 2004; Shinmura and Tanaka 2008); therefore, for the inhabitants of these regions, *M. papulosa* is an important food resource. Additionally, carrageenan has been isolated from some species of *Meristotheca*, such as *M. senegalensis* (Fostier et al. 1992) and *M. procumbens* (Prasad et al. 2005), and can be considered a viable source of this valuable bio-product.

Nevertheless, through intense harvesting and other anthropogenic activity, concern has been

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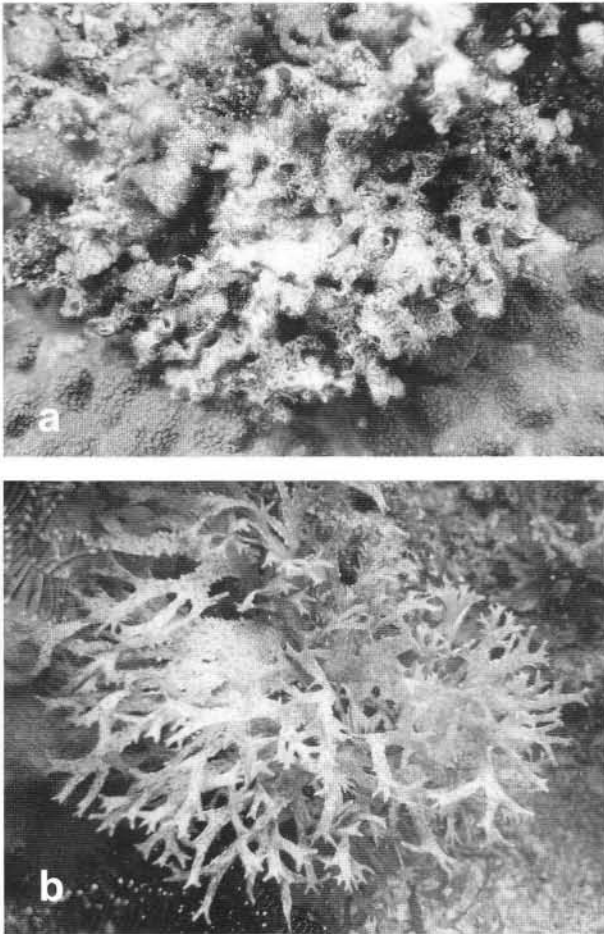


Fig. 1. *Meristotheca coacta* (a) and *M. papulosa* (b) in their natural habitat of Ushibuka (Amakusa-Shimojima Is.) and Cape Sata, respectively.

expressed regarding the reduction in the abundance of *M. papulosa*, as well as other similar species (e. g. Shinmura and Tanaka 2008). There remains a strong belief that stocks will continue to decline in the near future (Shinmura 2000; Makurazaki and Ohsumi-misaki Fisherman's Union, Kagoshima Prefecture, unpublished data). The importance of *M. papulosa* in Makurazaki and Ohsumi-misaki, is reinforced by the amount harvested from these areas, where 447,771 kg of biomass, with a value of 331.7 million yen, was harvested in 2000, and 302,860 kg, with a value of 195.8 million yen, was harvested in 2006. *Meristotheca coacta* is also widely found in the region, occurring simultaneously with *M. papulosa*. Hence, *M. coacta* is often taken as by-catch during *M. papulosa* harvests.

The intense harvesting steadily drives a decline in standing stock, however, habitat loss

may also be contributing to this decline. Habitat loss has often been linked with coastal pollution and coastal construction; however in the 21st century global climate change induced warming of coastal waters is also a possible factor. Indeed, climate change is driving increases in water temperature in many regions of the world (Domingues et al. 2008; Herr and Galland 2009). It is understood that changes in the geographic distribution should be expected for many species of plant and animals, including marine algae. Such shifts in distributions may lead to economic losses for local communities. Changes in water temperature of the East China Sea off the coast of Kyushu Island, Japan, have been recorded to have increased by $1.24 \pm 0.26^\circ\text{C}$ over a period of 1900 to 2010 (JMA 2011b). Changing environmental conditions can be expected to influence the harvest of these species.

Although there are a number of ecological and physiological studies regarding *Meristotheca*, the data presently available can only provide us with limited insight regarding the physiology of these macroalgae. Past research largely focuses on *M. papulosa*, and has examined how photosynthetic rates vary with depth by measuring changes in dissolved oxygen concentration (Yokohama 1973; Murase et al. 1989), in addition to how ultraviolet radiation influences their photobiology (Maegawa et al. 1993). The lack of physiological data regarding *M. coacta*, as well as *M. imbricata* remains conspicuous.

In the past, studies on the photobiology of *M. papulosa* have used manometric and electrochemical techniques (Yokohama 1973; Murase et al. 1989; Maegawa et al. 1989, 1993; Lideman et al. 2011). These studies provide results along a coarse temperature gradient, and relatively low intensities of irradiance. Nevertheless, municipalities in Ehime Prefecture have initiated cultivation of these species; however, commercial-scale operation remains elusive. One of the reasons for this lack of progress can be traced back to our limited understanding of their physiology. In this paper, we apply a quick and efficient technique, first developed to study photosynthesis in intact plants (pulse amplitude modulated (PAM)-chlorophyll fluorometry;

Aldea et al. 2006; Ralph et al. 2006; Kuster et al. 2007; Tsuchiya et al. 2012). We use this technology to provide detailed insight regarding the temperature response of *Meristotheca*, by using *M. coacta* and *M. papulosa* as experimental organisms, with the hope that this knowledge will help to advance cultivation of these species.

Materials and Methods

Specimen collection and stock maintenance

Meristotheca coacta and *M. papulosa* are widely distributed along the coast of southern Kyushu Is., Japan. Approximately 15 cm of fronds of the two species examined in this study were collected from different shores of Kyushu Island. Specimens of *M. coacta* were collected by SCUBA at Ushibuka town of Amakusa-Shimojima Is., Kumamoto Prefecture (32°11'N, 129°58'E) and *M. papulosa* were collected at

Ohtomari village of Cape Sata, Kagoshima Prefecture (31°01'N, 130°41'E) on 15 May 2010 (Fig. 2). *Meristotheca coacta* and *M. papulosa* were collected at water depth ranging from 1–5 m. Collected algae were stored in 500 ml plastic bottles with seawater and transported to the laboratory in a cooler at about 20°C. The specimens were maintained for 1 to 3 days before examination at the Faculty of Fisheries, Kagoshima University in an aquarium tank (2.0 × 1.0 × 0.5 m³) containing seawater at salinity of 33 PSU, pH of 8.0, water temperature of 20°C, and under photosynthetic active radiation (PAR) of 90 μmol photons m⁻² s⁻¹ (14:10 hours light: dark cycle).

Underwater temperature and PAR at the study sites

Underwater PAR was measured near the study sites. Off the coast of Cape Sata (31°30' N, 130°38'E), we took measurements from 12:30 and 13:00 on 2 July 2011 just below the seawater surface (0 m), and at depths of 3 m, 5 m, 10 m, 20 m, 30 m, 40 m and 50 m with light intensity data logger MDS-Mk-V/L (S/N200457, JFE-Advantech, Japan). The measurement was carried out every one second for one minute at the each depth. Underwater temperature was measured with light intensity by CTD (*T/S Nansei-maru*, Faculty of Fisheries, Kagoshima University). Additionally at Nagashima Is. (32°14'N, 130°9'E, which is near Amakusa-Shimojima Is.), measurements were taken from 11:30 and 12:00 on 7 July 2010 just below the seawater surface (0 m), and at 5 m, 10 m, 15 m, 20 m and 25 m depths by a PAR meter (LI-250 with spherical quantum sensor LI-193SA, Li-Cor, USA). The measurement was carried out every one second for thirty seconds at the each depth.

PAR measurements were used to determine the extinction coefficient (K) that fit the following equation (Beer-Lambert law):

$$I_D = I_0 \cdot \exp(-K \cdot D) \quad (1)$$

where, I_D is PAR at the some depth (D) in meters, I_0 is surface PAR coefficient, and K is the extinction coefficient

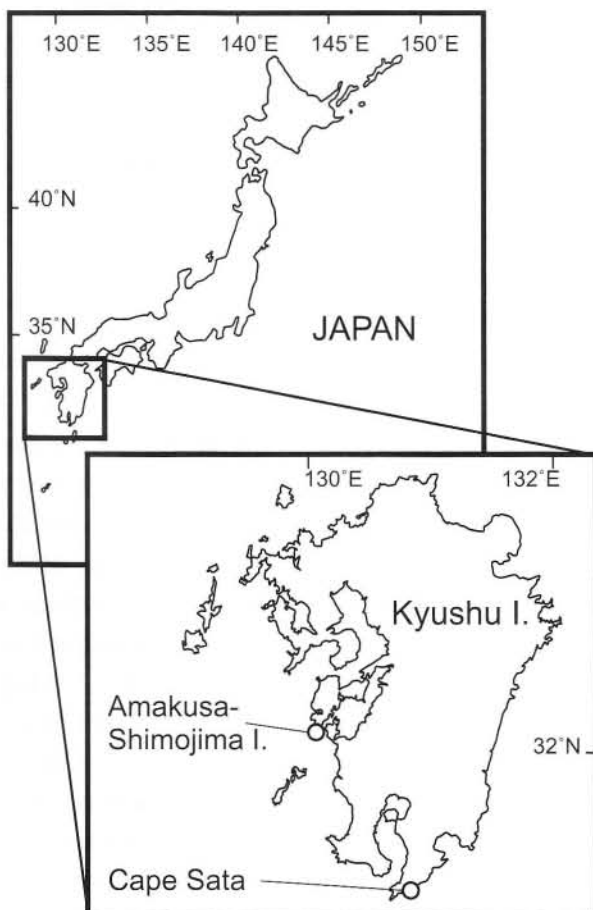


Fig. 2. Map of Kyushu Is., Japan showing the study sites of *Meristotheca coacta* and *M. papulosa*.

Rapid light curves (RLCs)

Rapid light curves (RLCs) were generated by running the standard algorithm of the pulse amplitude modulated (PAM)-chlorophyll fluorometer (Imaging-PAM, Heinz Walz GmbH, Germany) using an incremental sequence of actinic illumination periods, with light intensities increasing in 21 steps from 0 to 1,078 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ of PAR. Relative electron transport rate (rETR) was calculated using the equation:

$$rETR = 0.5 \cdot Y \cdot PAR \cdot AF \quad (2)$$

where, Y is the effective quantum yield of PSII ($\Phi_{\text{PSII}} = (F - F_m')/F_m'$, F is the initial fluorescence, and F_m' is 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 photosynthesis parameters

From each specimen, 2 cm long portions of the thalli were placed in a multi-well chamber (Falcon, USA) with sterilized seawater, allowing for 9 replicates for each species. Chamber temperature was controlled by a block incubator BI-535A (Astec, Japan) by placing the well-plate on the aluminum block of the incubator. Water temperature in the chamber wells were measured with a thermocouple in order to confirm that the water reached the desired temperature setting. The relative electron transport rates were determined by generating RLCs with 21 PAR levels over 20 minutes, for every 2°C increment temperature ranging from 8 to 34°C, hence once set RLC took 4 to 6 hours.

We modeled the rETR versus PAR to calculate the maximum rETR rate in the absence of photoinhibition (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 nonlinear model modified after Platt et al. (1980):

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

Based on these parameters, we can then

estimate the values of PAR_{sat} , which defines PAR when rETR begins to saturate (Eq. 3) and PAR_{opt} , which defines PAR when the rETR is at a maxima.

$$\frac{drETR}{dPAR} = \alpha \exp\left(-\frac{\beta}{\gamma} PAR - \frac{\alpha}{\gamma} PAR\right) - \beta \left(1 - \exp\left(-\frac{\alpha}{\gamma} PAR\right)\right) \exp\left(-\frac{\beta}{\gamma} PAR\right) \quad (4)$$

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 can be estimated from the first real root:

$$PAR_{\text{opt}} = \frac{\gamma}{\alpha} \ln\left(\frac{\alpha}{\beta} + 1\right) \quad (5)$$

by substituting PAR_{opt} into Eq. 3, we arrive at the value of rETR at the maxima ($rETR_{\text{max}}$) of the $P-I$ curve. Saturating PAR (PAR_{sat}) was calculated using the equation:

$$PAR_{\text{sat}} = \frac{rETR_{\text{max}}}{\alpha} \quad (6)$$

Statistical analysis

Statistical analyses were done using R (R Development Core Team 2011) and OpenBUGS (Thomas et al. 2006). To estimate the parameters of the nonlinear model (Eq. 2, 3 and 4), a two-level hierarchical Bayesian model was implemented using OpenBUGS, because maximum-likelihood and least-squares techniques did not converge to a solution. Uniform priors were defined for each hyperparameter in the model, and the parameters were then allowed to sample from the hyperparameter distributions. We ran 4 chains of 100,000 samples each, discarded the first half of each chain and thinned the results to obtain 1,000 samples for each chain (i.e., 4,000 samples of the posterior distribution). The relationship between the estimated parameters and experimental water temperature were also examined, using Generalized Linear Models (GLM) assuming a Gamma distribution for the model parameters (θ) and a linear (e.g., PAR_{opt} and PAR_{sat}) or log (e.g., α , β , γ , and $rETR_{\text{max}}$) link-function as appropriate. Two models were used to examine these relationships, a linear model, where $\theta \sim \text{species} + \text{temperature} + \text{species} \times \text{temperature}$ and a quadratic model, where $\theta \sim \text{species} + \text{temperature} + \text{species} \times \text{temperature} + \text{temperature}^2 + \text{species} \times \text{temperature}^2$.

Results

Underwater temperature and PAR at the study sites

Meristotheca coacta and *M. papulosa* was widely distributed along the coast of southern Kyushu Is. including our study sites: Cape Sata, Amakusa and Nagashima Is. Generally, both species were growing on the rocky substrata at depths between 3 to 30 m.

Underwater PAR measured offshore of Cape Sata, at the depths of 0 m to 50 m ranged from 2,143 to 11 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ on 2 July 2011. Near Nagashima Is., PAR at depths of 0 m to 25 m ranged from 248 to 10 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ on 7 July 2010 (Fig. 3), during the measurements, we experienced fine clear skies at Cape Sata; however, the skies were mostly cloudy at near Nagashima Is.. The Beer-Lambert equation was fitted to PAR measurements taken at the two study sites using a linear regression on the log-transformed PAR and was determined to be:

$$\text{Cape Sata: } I_{(D)} = 1,717 e^{-0.11 \cdot D} \quad (R^2 = 0.986)$$

$$\text{Nagashima: } I_{(D)} = 185 e^{-0.12 \cdot D} \quad (R^2 = 0.969)$$

where, the extinction coefficients (K) determined for waters near Cape Sata and Nagashima Is. were 0.11 and 0.12, respectively. The coefficient of surface PAR for the Cape Sata and

Nagashima Is. models were 1,717 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ and 185 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, respectively.

In general, maximum irradiance at the coastal area at noon was around 2,000 to 2,200 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$ during the study period (April to August). Underwater PAR, based on the parameters estimated at each location and assuming a surface irradiance of 2,000 (or 2,200) $\mu\text{mol photons m}^{-2} \text{s}^{-1}$, respectively, are provided in Table 1 for reference. At Cape Sata, estimated maximum irradiance of the habitat for the two species (ca. 3–30 m depth) ranged from 1,451 (1,596) to 81 (89) $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. For those of Nagashima Is. PAR ranged from 1,395 (1,535) to 55 (66) $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. It is relevant to note that water temperature measured offshore of Cape Sata, at the depths of 0 m to 50 m ranged from 24.9 to 18.2°C on 2 July 2011 (Table 1).

Rapid light curves (RLCs)

Unlike typical photosynthesis - irradiance curves that increase monotonically until reaching some asymptote, the rETR of these species were hump-shaped and expressed clear photo-inhibition at high PAR (Fig. 4). At any given temperature and PAR, the rETR of *M. coacta* tended to be higher than that of *M. papulosa*.

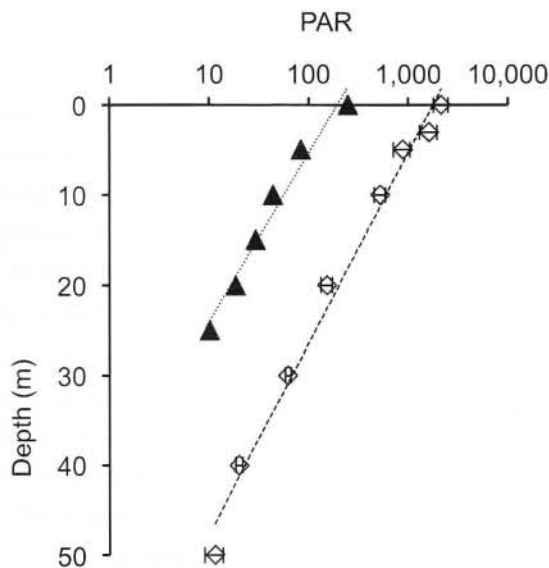


Fig. 3. Underwater photosynthetic active radiation (PAR, $\mu\text{mol photons m}^{-2} \text{s}^{-1}$) in Cape Sata (12:30 to 13:00, 2 July 2011) and Nagashima Is. (11:30 to 12:00, 7 July 2010).

Table 1. Underwater temperature at Cape Sata measured on 2 July 2011 and estimated-underwater PAR* at Cape Sata and Nagashima Is. if the surface irradiance was 2,000 or 2,200 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$

Depth (m)	Temperature (°C)		Estimated underwater PAR** ($\mu\text{mol photon m}^{-2} \text{s}^{-1}$)			
	Cape Sata	Cape Sata	Cape Sata		Nagashima	
			2,000	2,200	2,000	2,200
3	24.9	1,451	1,596	1,395	1,535	
5	24.1	1,171	1,288	1,098	1,207	
10	23.1	686	755	602	663	
20	21.2	235	259	181	200	
25	20.1	138	152	100	110	
30	19.4	81	89	55	60	
40	18.4	28	30	16	18	
50	18.2	9	10	5	5	

* Extinction coefficient was determined by measured data on 2 July 2011 (Sata) and 7 July 2010 (Nagashima).

** Cape Sata, $I_{(D)} = I_{(0)} e^{-0.11 \cdot D}$; Nagashima Island, $I_{(D)} = I_{(0)} e^{-0.12 \cdot D}$, $I_{(D)}$, PAR at the objective depth (m); $I_{(0)}$, PAR at the surface; D , objective depth (m).

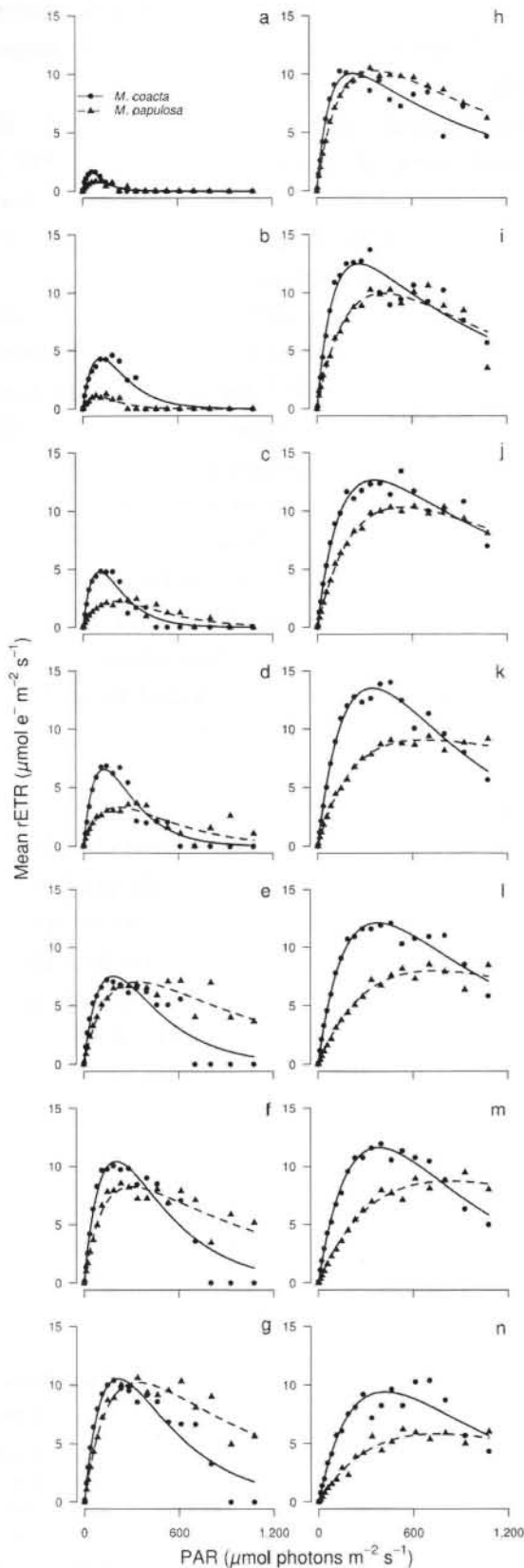


Fig. 4. The rapid light curves as determined by the hierarchical Bayesian analysis of *Meristotheca coacta* and *M. papulosa* determined over a temperature gradient of 8°C (a), 10°C (b), 12°C (c), 14°C (d), 16°C (e), 18°C (f), 20°C (g), 22°C (h), 24°C (i), 26°C (j), 28°C (k), 30°C (l), 32°C (m), 34°C (n). The solid and dash lines indicate the fitted model for *M. coacta* and *M. papulosa*, respectively. rETR, relative electron transport rate; PAR, photosynthetic active radiation.

By fitting Eq. 3 to the results using hierarchical Bayesian methods, we were able to elucidate the parameters of the model across all water temperatures, as well as derive estimates of PAR_{sat} , PAR_{opt} , and $rETR_{\text{max}}$. The parameters of the model as well as the derived estimates were then examined in detail using GLM (Fig. 5) to elucidate their dependence on temperature.

Temperature dependence of the photosynthetic model coefficients

The mean values of the maximum rETR in the absence of photoinhibition (γ), ranged from 14.0 to 47.0 $\mu\text{mol e}^- \text{m}^{-2} \text{s}^{-1}$ for *M. coacta* and 12.0 to 19.8 $\mu\text{mol e}^- \text{m}^{-2} \text{s}^{-1}$ for *M. papulosa* over the range of temperatures examined and did not appear to be related to temperature (Fig. 5a). The log-link gamma GLM fit to this data, revealed the insignificance of temperature \times species interactions ($F_{(1,24)}=0.0732$, $P=0.7891$) and of temperature dependence ($F_{(1,26)}=1.6425$, $P=0.2117$). However, a species effect was detected ($F_{(1,26)}=55.1018$, $P<0.0001$), indicating that that values for *M. coacta* were significantly higher than that of *M. papulosa*. There was an estimated 15.1 $\mu\text{mol e}^- \text{m}^{-2} \text{s}^{-1}$ difference in the parameter estimates of the maximum rETR rates among these species.

The mean values of the initial slope (α) of *M. papulosa* and *M. coacta* ranged from 0.023 to 0.093 $\mu\text{mol e}^- (\mu\text{mol photons})^{-1}$ and 0.065 to 0.156 $\mu\text{mol e}^- (\mu\text{mol photons})^{-1}$, respectively and were dome-shaped (Fig. 5b). Unlike the GLM for the parameter γ , a quadratic equation was fitted to α . Species effects were significant in the model ($F_{(1,26)}=209.3376$, $P<0.0001$), where α was greater for *M. coacta*. The value of α also significantly varied with the square of the temperature ($F_{(1,24)}=144.8558$, $P<0.0001$), which justifies the use of the quadratic model. For the quadratic case, the model can then be used to provide estimates of the maximum value of α and the temperature of its occurrence. In this case, maximal values occurred at 19.7°C and 20.8°C and were 0.148 $\mu\text{mol e}^- (\mu\text{mol photons})^{-1}$ and 0.078 $\mu\text{mol e}^- (\mu\text{mol photons})^{-1}$ for *M. coacta* and *M. papulosa*, respectively.

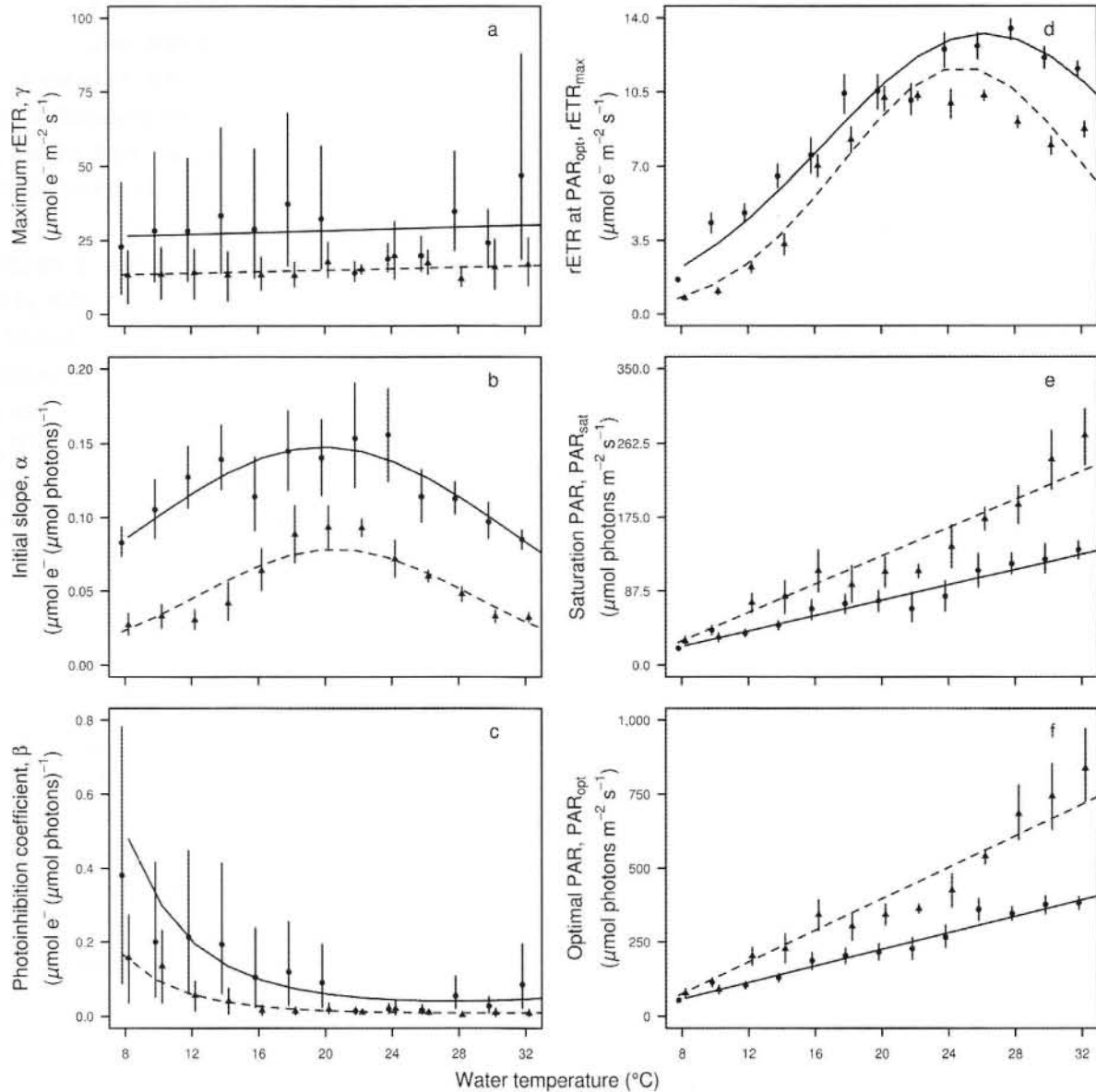


Fig. 5. The model parameters determined by the hierarchical Bayesian model express a variety of temperature dependence for both *Meristotheca coacta* (●) and *M. papulosa* (▲). (a) The maximum relative electron transport rates (rETR) in absence of photoinhibition, γ , are independent of temperature. (b) The initial slope of rapid light curves, α , can be described by a quadratic function of temperature. (c) The photoinhibition coefficient, β , can also be described by a quadratic function of temperature. (d) The maximum rETR that was observed when photosynthetic active radiation (PAR) reaches its optimal value, $rETR_{\max}$, can be described by a quadratic equation. (e) The PAR at which rETR rates begin to saturate, PAR_{sat} , is a linear function of temperature. (f) The PAR at which maximum rETR was observed, PAR_{opt} , is a linear function of temperature. The data are jittered about the experimental temperature to improve clarity and the bars indicate the 95% credible interval.

The mean values of the photoinhibition coefficient (β) of these species ranged from 0.014 to 0.381 $\mu\text{mol e}^- (\mu\text{mol photons})^{-1}$ for *M. coacta* and from 0.004 to 0.157 $\mu\text{mol e}^- (\mu\text{mol photons})^{-1}$ for *M. papulosa* and were U-shaped in nature (Fig. 5c). This parameter was also analyzed using the quadratic GLM, where the quadratic term was significant ($F_{(1,24)} = 19.7729$,

$P = 0.0002$). No interactions were evident ($F_{(1,23)} = 0.9393$, $P = 0.3430$), but species was an important factor in the model ($F_{(1,26)} = 37.8215$, $P < 0.0001$). A minima could be estimated near 27.6°C and 29.1°C for *M. coacta* and *M. papulosa*, respectively and were 0.041 $\mu\text{mol e}^- (\mu\text{mol photons})^{-1}$ for *M. coacta* and 0.009 $\mu\text{mol e}^- (\mu\text{mol photons})^{-1}$ for *M. papulosa*.

The mean values of $rETR_{\max}$ could also be examined using a quadratic model (Fig. 5d), and these values increased from low temperatures and peaked near 25.5°C. Indeed, the quadratic terms were significant ($F_{(1,24)} = 188.3459$, $P < 0.0001$) as was the species effect ($F_{(1,26)} = 25.2488$, $P < 0.0001$). Interactions among species and temperature was also significant ($F_{(1,23)} = 8.9195$, $P = 0.0068$). A more detailed examination of the model indicated that $rETR_{\max}$ peaked with a value of $13.3 \mu\text{mol e}^- \text{m}^{-2} \text{s}^{-1}$ at 26.0°C for *M. coacta* and was $11.7 \mu\text{mol e}^- \text{m}^{-2} \text{s}^{-1}$ at 25.1°C for *M. papulosa*.

Regarding the mean values for PAR_{sat} , which indicates the value of PAR when rETR began to saturate, they monotonically increased with increasing temperature (Fig. 5e). Indeed, temperature and species were significant factors in the differences determined for PAR_{sat} ($F_{(1,25)} = 339.128$, $P < 0.0001$ and $F_{(1,26)} = 80.949$, $P < 0.0001$) and there were significant interactions among temperature and species ($F_{(1,24)} = 37.383$, $P < 0.0001$). It is apparent that the PAR needed to saturate rETR was more sensitive to temperature and greater in magnitude for *M. papulosa*.

Similarly, the PAR where $rETR_{\max}$ was observed (at PAR_{opt}) monotonically increased with temperature (Fig. 5f), with a significant temperature effect ($F_{(1,25)} = 435.62$, $P < 0.0001$) and a significant species effect ($F_{(1,26)} = 112.09$, $P < 0.0001$). There were also significant interactions describing the relationship between species and temperature ($F_{(1,24)} = 56.66$, $P < 0.0001$), where PAR_{opt} for *M. papulosa* was generally higher and more sensitive to temperature, compared to *M. coacta*.

Discussion

In our study, the initial slope (α) of *M. papulosa* and *M. coacta* showed higher values at temperatures from 18 to 28°C (Fig. 5b). Meanwhile, the photoinhibition coefficient (β) of the two species decreased from low temperatures (Fig. 5c), and $rETR_{\max}$ increased from low temperatures to a peak between 25 to 26°C (Fig. 5d). This result suggests that the optimal temperature

of two species is most likely within the range of 18 to 28°C, and corresponds well to an earlier study of *M. papulosa* that examined dissolved oxygen production and respiration rates (Lideman et al. 2011), and are well within the range of water temperatures observed in their natural habitat.

More specifically, we can define a range of temperatures that are optimal for the photosynthetic activity of these species based on the model results. Let the optimal temperature range for some parameters be defined as the parameter (e.g., values of α , β , and $rETR_{\max}$) estimates that are at least 95% of the estimated maximum or minimum parameter values. Hence, for $rETR_{\max}$, 95% of the maximum would be $12.6 \mu\text{mol e}^- \text{m}^{-2} \text{s}^{-1}$ for *M. coacta* and $11.1 \mu\text{mol e}^- \text{m}^{-2} \text{s}^{-1}$ for *M. papulosa*, which leads to temperature range of 22.9–29.1°C and 22.7–27.4°C, respectively. Similarly, for α the temperature ranges can then be determined, which were 16.1–23.3°C for *M. coacta* and 18.2–23.4°C for *M. papulosa*. In the case of β , we examine the values that are at least 95% of the parameter minima, therefore temperatures ranged from 24.8–30.3°C for *M. coacta* and 26.3–31.8°C for *M. papulosa*. However, these estimates are for individual parameters, therefore we must combine this information to produce a general estimate of optimal temperature range. Hence, let the optimal temperature range be the range of temperatures that are the union of the temperature ranges determined for each of the parameters. This reveals that the optimal temperature range for *M. coacta* is 16.1 to 30.3°C and for *M. papulosa*, it is 18.2 to 31.8°C.

Regarding their response to PAR, we observed inhibitory effects at high irradiances, based on the RLC determined at each temperature and for each species, adding much needed information to earlier studies, such as Lideman et al. (2011), which only examined $PAR < 600 \mu\text{mol photons m}^{-2} \text{s}^{-1}$. The initial slope (α) of *M. coacta* was always higher than that of *M. papulosa* at each temperature condition examined (Fig. 5b). However, the PAR_{sat} and PAR_{opt} for the former were always lower than those of the latter (Fig. 5f), suggesting that *M. coacta* can

photosynthesize and survive under lower levels of PAR. Perhaps this difference is related to their habitats, because the prostrated appearance of *M. coacta*, found growing on the rocks, is sometimes shaded by *M. papulosa* and other organisms (Fig. 1).

The experiments on *M. coacta* and *M. papulosa* demonstrated that optimal temperatures were typically of values observed in the field, where temperatures were 20 to 25°C. This was expected given that photosynthetic performance is one of the most important processes that drives the life-cycle of photosynthetic organisms. The close correlations between laboratory-derived estimates of optimal temperature and the field-temperature of the habitats of marine algae are well demonstrated in a variety of species and among phyla. Nishihara et al. (2004) has shown that the red alga *Laurencia brongniartii* J. Agardh performs optimally at temperature ranging from 22 to 28°C, which is also within typical values of water temperature observed in its preferred coral reef habitat. Ohno et al. (1994) demonstrated that *Kappaphycus alvarezii* (Doty) Doty ex Silva from subtropical waters of Japan also grew well at temperatures between 25 and 28°C. More relevantly, the photosynthetic parameters of *Gracilaria cornea* J. Agardh (= *Hydropuntia cornea* (J. Agardh) Wynne) was optimal at temperature of 25°C (Dawes et al. 1999), which is with the range of our results for subtropical red algae species.

Species was a significant factor influencing the relationship between the parameters of the GLM models with respect to temperature, suggesting that the responses to temperatures are species specific. However, it is important to note that maximal rates of rETR (i.e., $rETR_{max}$) for each species occurred at roughly similar temperatures with wide standard errors. This may partially explain why they are often found together in the intertidal zone. It is also important to note that the β of *M. coacta* was higher than *M. papulosa* especially at higher temperatures, suggesting that *M. papulosa* is less susceptible to high PAR in warmer waters. Perhaps, this can partly explain the presence

of *M. papulosa* in regions of Africa, Southwest Asia, China, Southeast Asia, Australia and New Zealand, and the prevalence of *M. coacta* in Japan (Yoshida 1998), Korea (Lee and Kang 2001), Taiwan (Huang 2000) and the Philippines (Kraft et al. 1999).

By modeling the *P-I* curve and the relationship between the estimated parameters and temperature, the response of these organisms over the range of experimental temperatures can be predicted. This is important, since the development of protocols and cultivation systems require the appropriate models as input. The results of this study can be used as the base to develop highly optimized design equations that will maximize production while minimizing costs at the commercial scale.

The analysis of the experiments provided us with a range of temperatures that were optimal for maximum photosynthetic activity. These temperatures correspond well to those determined in the natural habitat, which is reassuring given that discrepancies between experimental results and field data are not uncommon (Lobban and Harrison 1997). However, it should be noted that there was a mismatch between PAR measured *in situ* and PAR that maximized rETR, which will require further investigation (Tsuchiya et al. 2012). Models describing the rETR performance of *M. coacta* and *M. papulosa* and the temperature dependence of the model parameters should help to accelerate the cultivation of these species by fine-tuning the cultivation strategies used for these economically important red algae.

PAR_{sat} and PAR_{opt} value of *M. coacta* and *M. papulosa* (Figs. 5e, 5f) measured in this study increased with increasing of water temperature (Collins and Boylen 1982; Palmisano et al. 1987; Henley 1992, 1993). We suggest that if the water temperature increases, these species may be able to grow more effectively in the shallow waters of their environment, rather than in deeper water. As a sublittoral algal species, *M. coacta* and *M. papulosa* required PAR with a wider range compared, to Lüning (1981), which suggested that in the upper and mid-sublittoral, algae species only require light

ranging from 150 to 250 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$. Indeed, saturating irradiances show some correlation with habitat, but generally they are low compared to full sun (Reiskind et al. 1989). Moreover, above the saturation point (PAR_{sat}), the light-dependent reactions are producing more ATP and NADPH that can be used by the light-independent reaction for CO_2 fixation, and therefore, increasing irradiance no longer causes any increase in photosynthetic rate (i.e., full saturated) (Barsanti and Gualtieri 2006).

Meristotheca coacta and *M. papulosa* generally can be found at the depth from 3 to 30 m deep. In this study, estimated maximum PAR at a depth of 30 m (Fig. 3, Table 1) corresponded to the mean values for the PAR_{sat} estimated at the temperatures from 18 to 22°C (Fig. 5e). These temperatures also corresponded to the temperatures measured at the depth of the study site (Table 1). We believe that the low value of the extinction coefficients is one mechanism that enables the success of these species in sublittoral waters.

Additionally, Tsuchiya et al. (2011) reported that the seasonal changes of seawater temperature near the study site (Kagoshima Bay) in 2009 and 2010 ranged from 15.6°C in February to 29.4°C in August. Especially, the temperature in April to August were recorded 18 to 28°C. Indeed, from 2006 to 2010 offshore of the study site, average monthly surface temperatures in April to August were also recorded to be from 18 to 28°C by JMA (2011b). Increasing temperatures as a result of global warming (JMA 2011a) may lead these macroalgae to change in spatial distribution in the future, because of the interactive links between PAR and water temperature on photosynthetic activity, given that these physical variables are one of the most important abiotic factors influencing the distribution of marine species (Lalli and Parsons 1997). It is important to note that in this region, the average winter and seawater temperatures have increased by about 1.1 and 0.7°C, respectively over the last 38 years (Tsuchiya et al. 2011). How this will affect the distribution of these economically important species remains to be determined.

Hence, we must diligently monitor the changing environment, because although these two edible seaweeds, *M. coacta* and *M. papulosa*, are currently adapted to the natural light and temperature circumstances of southern Kyushu Is., Japan, changing water temperatures may have a drastic effect on their distribution. Furthermore, the models determined from this study should greatly contribute to the design and management of mariculture programs and cultivation systems. Based on our results we suggest that either of the species can be successfully cultivated from April to August in this region.

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食用海藻 2 種、キクトサカとトサカノリ（紅色植物門ミリン科）の 光合成活性における光と温度の影響

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食用海藻であるキクトサカとトサカノリ（紅色植物門ミリン科）の生理特性を把握するために、様々な温度と光の条件での光合成活性を測定し、2種の動態を考察した。測定にはパルス変調クロロフィル蛍光測定法（Imaging-PAM）を用い、8-34℃の間の14温度条件と、光量 0-1,078 $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ の間の21条件の組合せで電子伝達速度（rETR）を測定した。初期勾配（ α ）、光阻害（ β ）、係数（ γ ）は2段階階層ベイズモデルを用いて非線形の光曲線モデルを得た。モデルで見積もられた至適な光合成活性は両種とも18-28℃の温度範囲で得られ、光合成に至適な光量（ PAR_{opt} ）は温度の増加と共に上昇した。また、両種は九州南部での生育地・水深における光や温度によく適応していた。これらの結果は海面養殖等における環境条件の検討に有効と考えられた。