

## ***In Vitro* Growth and Photosynthesis of Three Edible Seaweeds, *Betaphycus gelatinus*, *Euclidean serra* and *Meristotheca papulosa* (Solieriaceae, Rhodophyta)**

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**Abstract:** Three species of Solieriaceae (Rhodophyta), *Betaphycus gelatinus*, *Euclidean serra* and *Meristotheca papulosa* are economically important species, that are found in subtropical to tropical waters and are well represented in the coastal area of Ryukyu and Kyushu Islands of Japan. This study was motivated by the need to establish basic information regarding their physiology, in order to design a cultivation system. Two experiments were conducted to determine how five temperature treatments of 16, 20, 24, 28 and 32°C influenced growth and photosynthesis, as determined by a dissolved oxygen sensor. An additional experiment examined how nine irradiance levels of 0, 13, 26, 48, 68, 168, 248, 342 and 536  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  at 24°C influenced photosynthetic rates. Optimal relative growth rates of *B. gelatinus* and *E. serra* under 90  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  occurred from 24°C and 28°C, while for *M. papulosa* it ranged from 20°C and 24°C. Maximum photosynthetic rates ( $P_{\text{max}}$ ) for *B. gelatinus*, *E. serra* and *M. papulosa* were 135.0, 65.0 and 52.4  $\mu\text{g O}_2$  (mg chl-a)<sup>-1</sup> min<sup>-1</sup>, respectively and saturated beyond 94.9, 69.4 and 35.4  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ , respectively. These characteristic results were closely related to their depth of the habitat and local distribution in southern Japan, and provide information required to design their mass-cultivation systems.

**Key words:** *Betaphycus gelatinus*; *Euclidean serra*; *Meristotheca papulosa*; Photosynthesis

The pan-tropical/temperate red algal family, Solieriaceae (Gigartinales), includes many economically important species, of which three genera, *Euclidean*, *Betaphycus* and *Kappaphycus*, which are actively harvested in the Philippines and other Southeast Asian countries (Parker 1974; Trono 1993) are regarded as one of the major sources of carrageenan in the world (Santos 1989; Glenn and Doty 1990). Species of these genera are also well represented along the shores of southern part of Japan (Yoshida 1998), with seven species recognized for the region (Yoshida and Yoshinaga 2010). Meanwhile, other taxa in the Solieriaceae, *Meristotheca*, are also distributed in Japan, and three species have been

reported.

Traditionally, these Japanese species have been used as the ingredients for soup and salad in the Ryukyu and Kyushu Islands, southern Japan (Ohno 2004; Shinmura and Tanaka 2008). In these regions, *Betaphycus gelatinus* (Esper) Doty ex Silva (Fig. 1a), *Euclidean serra* (J. Agardh) J. Agardh (Fig. 1b) and *Meristotheca papulosa* (Montagne) J. Agardh (Fig. 1c) can be regarded as important regional resources. Notably, *E. serra* and *M. papulosa* are mainly harvested in southern Kyushu Is. (Kagoshima and Miyazaki Prefectures), and *B. gelatinus* is harvested in Ishigaki and Miyako Is. of Okinawa Prefecture. All are indispensable to

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**Fig. 1.** Species of Solieriaceae in their natural environment used in this experiment. a, *Betaphycus gelatinus*; b, *Eucheuma serra*; c, *Meristotheca papulosa*.

the local dietary culture of these regions. However, as a result of anthropogenic activity, there is a great possibility that these resources will decline, especially for economically important and actively harvested species such as *M. papulosa* (Shinmura 2000; Makurazaki and Ohsumi-misaki Fisherman's union yield data unpublished) as well as other species (e.g., Shinmura and Tanaka 2008).

Ecological and physiological studies of *Eucheuma* and 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 enhance of cultivation techniques and supply. However, there is little knowledge for Japanese taxa, which could lead to innovations in cultivation. Studies to date have examined the yield and characteristics of lectins (Kawakubo et al. 1997, 1999), photosynthesis versus irradiance (*P-I*) characteristics of *Meristotheca papulosa* (Yokohama 1973; Murase et al. 1989; Maegawa et al. 1993), experimental tank culture studies (Ohno et al. 2002), and the effects of water motion on nutrient supply (Nishihara and Terada 2010). Currently, no commercial-sized *Eucheuma sensu lato* (including *Kappaphycus* and *Betaphycus*) / *Meristotheca* cultivation systems has been established in Japan. Although a trial experiment of *M. papulosa* aquaculture was started by the Government of Ehime Prefecture (unpublished), its system is still incomplete. We suggest that optimum temperature and light conditions, and growth characteristics must be

elucidated so that we can innovate advanced cultivation technology, strategy, and conserve natural populations.

In this study, we focused on elucidating the photosynthesis and growth response of the three edible seaweeds, *B. gelatinus*, *E. serra* and *M. papulosa* from Ryukyu and Kyushu Islands, southern Japan, by manipulating light and temperature conditions.

## Materials and Methods

### *Specimen collection and culture*

The three species examined in this study were collected from different shores. Specimens of *B. gelatinus* (Fig. 1a) were collected from Yoshiwara, Ishigaki Island, Okinawa Prefecture (24°27'25.07"N, 124°09'20.00"E; Depth: 2 m) on 26th December, 2007. *Meristotheca papulosa* (Fig. 1b) and *Eucheuma serra* (Fig. 1c) were collected in Kagoshima Prefecture at Makurazaki (31°15'58.84"N, 130°17'24.14"E; Depth: 10 m) on 9th May, 2008 and at Ohtomari near Cape Sata (31°01'14.60"N, 130°41'09.33"E; Depth: 5 m) on 1st June, 2008, respectively. Each sample was collected by SCUBA and skin diving by the authors or local fisherman. Collected algae were stored in 500 ml seawater and transported to the laboratory in a cooler at approximately 20°C. The specimens were maintained at the Faculty of Fisheries, Kagoshima University, Japan in an aquarium tank (2.0 × 1.0 × 0.5 m<sup>3</sup>) containing seawater of salinity at 33 PSU, pH of 8.0, 90 μmol photon m<sup>-2</sup> s<sup>-1</sup> and 20°C under 14:10 light: dark cycle. Temperature of the aquarium tank was decided on the basis of natural seawater temperature at the collecting date.

### Monitoring of the growth

Culture studies were carried out using the modified procedures of Terada and Yamamoto (2000) and Nishihara et al. (2004a, 2004c). Before conducting the culture experiment, 1.0 to 1.5 cm sections of the thallus of each macroalga was cleaned to remove any attached epiphytes, and microscopic organisms were removed by brushing under a microscope. Cleaned specimens were transferred to a 50 ml screw bottle containing the half concentration (1/2) of Provasoli's Enriched Seawater (PES) medium (Anderson 2005) with  $\text{GeO}_2$  (10 mg/l), 33 PSU and pH of 7.8. These cultures were grown in an incubator (MLR-351, Sanyo Electronic Co. Ltd., Tokyo) for 2 weeks under  $90 \mu\text{mol photon m}^{-2} \text{s}^{-1}$  (fluorescent lamp) and a 12L: 12D light cycle to establish unialgal culture. After 2 weeks acclimatization, cultured explants free from contaminants were used for experiment.

Five temperature treatments with five replicates were examined at 16, 20, 24, 28 and  $32^\circ\text{C}$  with the same fluorescent lamp at the same light irradiance ( $90 \mu\text{mol photon m}^{-2} \text{s}^{-1}$ ) to examine the effects of temperature on the growth of *B. gelatinus*, *E. serra* and *M. papulosa*, respectively. Nutrient concentrations and other physical parameters were similar to those used during creation of explants. These cultures were maintained in the Multi Thermo Incubator (MTI-201, EYELA, Tokyo Rikakikai Co., Ltd., Tokyo) for 20 days. Thalli weights were measured every 4 days using an electronic balance (Mettler Toledo AG 204, Ohio). Culture media were changed every 8 days.

Weight gain (%) and relative growth rate (RGR, %  $\text{day}^{-1}$ ) were calculated over the exponential growth phase using the following equations:

$$\text{Weight gain} = [(W_1 - W_0) / W_0] \times 100 \quad (1)$$

$$\text{RGR} = [(\ln W_1 - \ln W_0) / (t_1 - t_0)] \times 100 \quad (2)$$

where,  $W_0$  and  $W_1$  are the explants weight at time  $t_0$  and  $t_1$ , respectively.

### Experimental condition of temperature effect on the photosynthesis

Five temperature treatments of 16, 20, 24, 28 and  $32^\circ\text{C}$  with four replicates were examined at an irradiance of  $248 \mu\text{mol photon m}^{-2} \text{s}^{-1}$  that was higher than saturation irradiance ( $I_k$ ) determined from the photosynthesis versus irradiance (*P-I*) experiment, and provided by a metal-halide lamp (Nishihara et al. 2004a). Irradiance was measured with the LI-193SA (with LI-250) underwater quantum sensor (Li-Cor Inc., Nebraska). Temperature was adjusted and maintained by a temperature controlled water bath (Coolnit CL-80F, Taitec, Inc., Tokyo). Photosynthetic rates were determined by measuring the dissolved oxygen concentration (mg/l) every 5 minutes for 30 minutes after pre-incubation for 30 minutes acclimatization at each experimental condition. Dissolved oxygen (DO) was measured by using a polarographic probe and a DO meter (Model 58, and 5100, YSI Incorporated, Ohio). Explants used in this experiment for *B. gelatinus*, *E. serra* and *M. papulosa* were approximately 13, 7 and 75 mg wet weight ( $\text{mg}_{\text{ww}}$ ), respectively. Explants were cut from the plants in the aquarium tank, and were acclimatized overnight with sterilized seawater in the incubator (Muraoka et al. 1998; Serisawa et al. 2001). To start the experiment, they were placed in BOD bottles containing 99.2 ml sterilized seawater. The DO sensors were placed in the sterilized seawater in a manner so that there no visible bubbles occurred. Seawater was continuously stirred during the measurement.

### Experimental condition of irradiance effect on the photosynthesis

Photosynthetic rates were determined at eight treatments of irradiance at 13, 26, 48, 68, 168, 248, 342 and  $536 \mu\text{mol photon m}^{-2} \text{s}^{-1}$  with four replicates in  $24^\circ\text{C}$ . As with temperature, photosynthetic rates were determined every 5 minutes for 30 minutes by measuring the DO concentration (mg/l). Methods used for this experiment follow those of the temperature experiment.

The *P-I* parameters for each temperature



were determined by using a nonlinear least squares regression of the following equation:

$$P_{\text{net}} = (P_{\text{max}} \times \tanh [\alpha / P_{\text{max}} \times I]) + R_{\text{d}} \quad (3)$$

where,  $P_{\text{net}}$  was the net  $\text{O}_2$  production rate,  $P_{\text{max}}$  was the maximum  $\text{O}_2$  production rate,  $\alpha$  was the initial slope of the photosynthesis versus irradiance curve,  $I$  was the incident irradiance, and  $R_{\text{d}}$  was the dark respiration rate (Jassby and Platt 1976; Henley 1993). Saturation irradiance ( $I_k$ ) was calculated as  $P_{\text{max}} / \alpha$  and compensation irradiance ( $I_c$ ) was  $R_{\text{d}} / \alpha$  (Schubert et al. 2006).

#### Measurement of chlorophyll-*a*

Samples were extracted under refrigeration by using 10 ml N, N-dimethylformamide solvent for 24 hours, and the absorbance (Abs) was determined using a spectrophotometer (UV-1600, Shimadzu Corporation, Tokyo) at 700 nm, 646.8 nm and 663.8 nm. Chlorophyll-*a* (chl-*a*) was calculated according to Porra et al. (1989) by using the following equation:

$$\text{chl-}a \ (\mu\text{g ml}^{-1}) = 12.0 \times (\text{Abs}_{663.8} - \text{Abs}_{700}) - 3.11 \times (\text{Abs}_{646.8} - \text{Abs}_{700}) \quad (4)$$

where  $\text{Abs}_{663.8}$  was the absorbance at 663.8 nm,  $\text{Abs}_{646.8}$  was the absorbance at 646.8 nm and  $\text{Abs}_{700}$  was the absorbance at 700 nm.

#### Statistical analysis

A one-way ANOVA ( $\alpha < 0.05$ ) was used to examine how the temperature affected the growth rate and photosynthetic rate by using the software Super-ANOVA. The Duncan new multiple range test was selected to identify significantly different means among the temperatures.

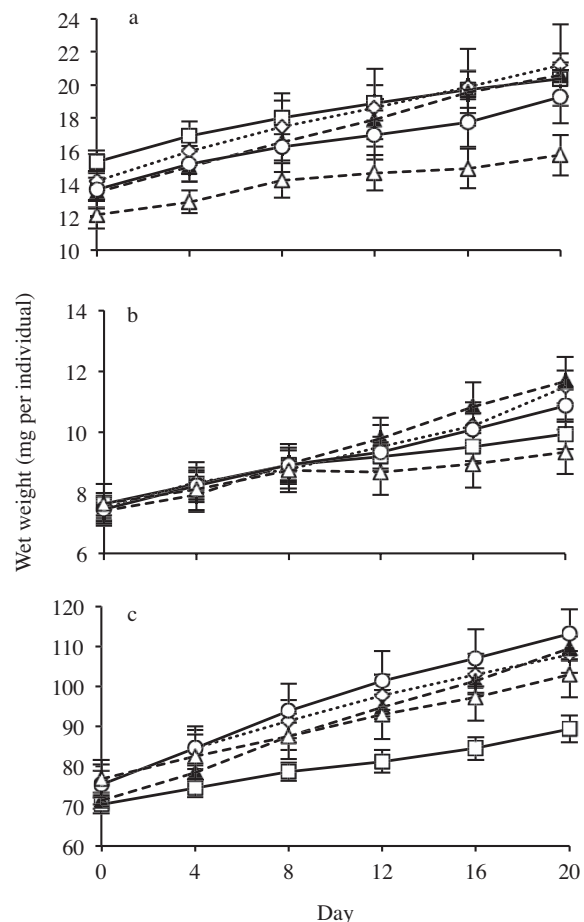
## Results

#### Effect of temperature on the growth

Weight of explants increased steadily during the 20 days of laboratory cultures, however, explants of *M. papulosa* cultured at 32°C lost a little of their characteristic red pigmentation at the end of the experiment (Fig. 2). Initial weight of *B. gelatinus* was around 14 mg. After 20-days

culture, the maximum weight was observed at 24°C (52.9 mg), and the lowest was 16°C (29.4 mg). In case of *E. serra*, initial weight was around 7 mg. After the culture period, the maximum weight was observed at 24°C (57.6 mg), and the lowest was 16°C (22.3 mg). Meanwhile, initial weight of *M. papulosa* was around 73 mg. After 20-days culture, the maximum weight was observed at 24°C (109.4 mg), and the lowest was 32°C (89.3 mg).

Maximum weight gain of *B. gelatinus*, *E. serra* and *M. papulosa* occurred at the same temperature of 24°C and after 20 days of culture, they were 52.9, 57.6 and 53.2%, respectively (Table 1). The lowest weight gain for *B. gelatinus* and *E. serra* were observed at 16°C with rate of 29.4 and 22.3%, respectively, while for *M. papulosa* occurred at 32°C with rate of

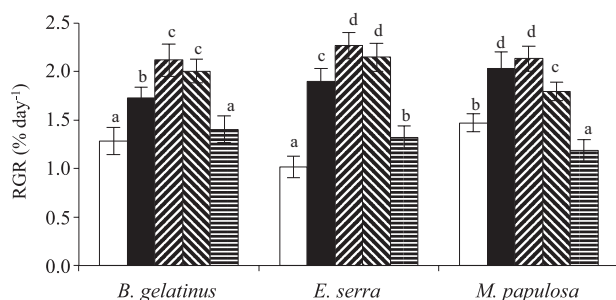


**Fig. 2.** *In vitro* growth of *Betaphycus gelatinus* (a), *Eucheuma serra* (b) and *Meristotheca papulosa* (c) at the five different temperature levels in 20-days cultures ( $\triangle$ , 16°C;  $\circ$ , 20°C;  $\blacktriangle$ , 24°C;  $\diamond$ , 28°C;  $\square$ , 32°C). Bars show the standard deviation.

**Table 1.** Weight gain (%) of *Betaphycus gelatinus*, *Euclidean serra* and *Meristotheca papulosa* at the five different temperature levels after 20-days *in vitro* cultures

	16°C	20°C	24°C	28°C	32°C
<i>B. gelatinus</i>	29.4 ± 3.7	41.3 ± 3.2	52.9 ± 3.7	49.4 ± 3.7	32.4 ± 3.6
<i>E. serra</i>	22.3 ± 2.7	46.0 ± 4.0	57.6 ± 4.3	53.5 ± 4.5	30.2 ± 3.1
<i>M. papulosa</i>	34.1 ± 2.6	49.9 ± 4.9	53.2 ± 3.9	43.2 ± 2.8	26.9 ± 2.9

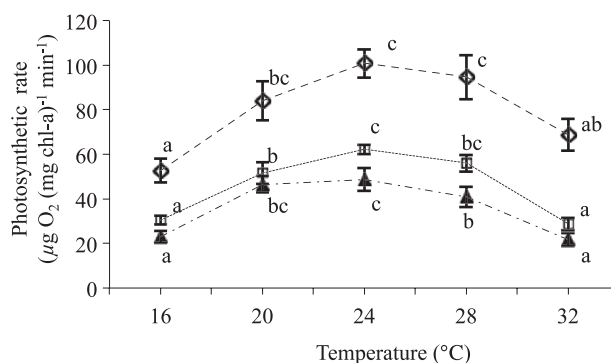
Errors show the standard deviation (n=5).



**Fig. 3.** Relative growth rate (RGR) of *Betaphycus gelatinus*, *Euclidean serra* and *Meristotheca papulosa* at the different temperature levels in 20-days cultures (□, 16°C; ■, 20°C; ▨, 24°C; ▩, 28°C; ▪, 32°C). Bars show the standard deviation. Means with different alphabet (a, b, c, d) are significantly different ( $P < 0.05$ ).

26.9%. Statistically, there were significant differences ( $P < 0.05$ ) among temperature levels (16, 20, 24, 28 and 32°C) for each species of *B. gelatinus*, *E. serra* and *M. papulosa*.

Maximum RGR of *B. gelatinus* was 2.1% day<sup>-1</sup> at 24°C followed by 2.0% day<sup>-1</sup> at 28°C (Fig. 3). However, these RGR were not significantly different ( $P > 0.05$ ) from each other. The RGR at 20°C and 32°C with value of 1.7 and 1.4% day<sup>-1</sup>, respectively was significantly lower compared to 24°C and 28°C. The lowest RGR of *B. gelatinus* was 1.3% day<sup>-1</sup> at 16°C and did not significantly differ with RGR at 30°C. The highest RGR of *E. serra* was 2.3% day<sup>-1</sup> at 24°C, but was not significantly different with RGR at 28°C with rate of 2.2% day<sup>-1</sup>. Moreover, the RGR significantly decreased ( $P < 0.05$ ) to 1.9% day<sup>-1</sup> at 20°C, followed by 1.3 and 1.0% day<sup>-1</sup> at 32°C and 16°C, respectively. In the case of *M. papulosa*, maximum RGR was 2.1% day<sup>-1</sup> occurring at 24°C, and it was not significantly different ( $P < 0.05$ ) with RGR at 20°C with a value of 2.0% day<sup>-1</sup>. The RGR value decreased significantly ( $P < 0.05$ ) at temperature level of 16, 28, and 32°C with value 1.8, 1.5 and 1.2% day<sup>-1</sup>, respectively.



**Fig. 4.** Photosynthesis versus temperature ( $P$ - $T$ ) curve of *Betaphycus gelatinus* (◇), *Euclidean serra* (□) and *Meristotheca papulosa* (▲) at the different temperature. Bars show the standard deviation. Means with different alphabet (a, b, c) are significantly different ( $P < 0.05$ ).

#### Effect of temperature on the photosynthetic rate

Photosynthetic rates of three macroalgae based on chl-a are presented in Fig. 4. Photosynthetic rates gradually increased by increasing the temperature from 16 to 24°C, and further decreased by increasing the temperature to 28 and 32°C. The highest photosynthetic rates were observed in *B. gelatinus*. There were significant differences ( $P < 0.05$ ) among five temperature treatments on photosynthetic rate in *B. gelatinus*. The highest photosynthetic rate of *B. gelatinus* was 101 µg O<sub>2</sub> (mg chl-a)<sup>-1</sup> min<sup>-1</sup> at 24°C and was not significantly different with photosynthetic rates at 20 and 28°C. Furthermore, the lowest photosynthetic rate was 52.7 µg O<sub>2</sub> (mg chl-a)<sup>-1</sup> min<sup>-1</sup> found at 16°C (Fig. 4).

As for *M. papulosa*, increases in temperature from 16°C to 24°C increased the photosynthetic rate from 22.9 to 48.8 µg O<sub>2</sub> (mg chl-a)<sup>-1</sup> min<sup>-1</sup> and dropped to 21.7 µg O<sub>2</sub> (mg chl-a)<sup>-1</sup> min<sup>-1</sup> at 32°C. Maximum photosynthetic rate at 24°C was not significantly ( $P > 0.05$ ) different with rate at 20°C (Fig. 4).

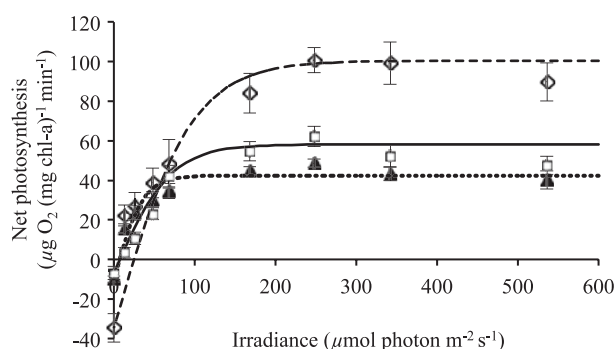
Similarly, the highest photosynthetic rate of *E. serra* was also found at 24°C with rates of

62.2  $\mu\text{g O}_2$  (mg chl-a) $^{-1}$  min $^{-1}$ , followed by 56.0, 51.5 and 30.4  $\mu\text{g O}_2$  (mg chl-a) $^{-1}$  min $^{-1}$  at 28, 20 and 16°C, respectively. The lowest photosynthetic rate was 28.8  $\mu\text{g O}_2$  (mg chl-a) $^{-1}$  min $^{-1}$  observed at 32°C. The highest photosynthetic rate at 24°C was not significantly different with the photosynthetic rate at 28°C ( $P > 0.05$ ), but significantly different with other treatments ( $P < 0.05$ ) (Fig. 4).

#### Effect of irradiance on the photosynthetic rate

Photosynthetic rates could be modeled with the hyperbolic tangent form of the  $P-I$  equation (Fig. 5). Respiration rates ( $R_d$ ) of *B. gelatinus*, *E. serra* and *M. papulosa* was 34.6, 6.8 and 10.0  $\mu\text{g O}_2$  (mg chl-a) $^{-1}$  min $^{-1}$ , respectively. Photosynthetic rates then increased sharply from 13 to 168  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  then increased slowly until 248  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ . Maximum photosynthetic rates of *B. gelatinus*, *E. serra* and *M. papulosa* was 100.8, 62.2 and 48.8  $\mu\text{g O}_2$  (mg chl-a) $^{-1}$  min $^{-1}$ , respectively, and all photosynthetic rates reached their maximal values by 248  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ .

Regarding the parameters of the  $P-I$  model,



**Fig. 5.** Photosynthesis versus Irradiance ( $P-I$ ) curve model of *Betaphycus gelatinus* ( $\diamond$ ), *Eucheuma serra* ( $\square$ ) and *Meristotheca papulosa* ( $\blacktriangle$ ). Bars show the standard deviation.

$P_{\max}$  for *B. gelatinus*, *E. serra* and *M. papulosa* was 135.0, 65.0 and 52.4  $\mu\text{g O}_2$  (mg chl-a) $^{-1}$  min $^{-1}$ , respectively; saturating irradiance ( $I_k$ ) occurred at 94.9, 69.4 and 35.4  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ , respectively and compensation irradiance ( $I_c$ ) occurred at 24.3, 7.3 and 6.7  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ , respectively (Table 2).

## Discussion

The successful cultivation of macrophytes, such as the three species we examined in this study, requires the detailed elucidation of their physiological response to environmental variables that are commonly under the control of the aquaculturist. Our study clearly shows the effects of temperature and irradiance on the physiology of *B. gelatinus*, *E. serra* and *M. papulosa* and provides fundamental knowledge of the growth and photosynthetic parameters that can be used to develop cultivation systems and methodologies, which may lead to the sustained availability of high quality products and the protection of natural populations. Especially, the range for optimum temperature and irradiance will contribute to the design and management of mariculture programs and tank cultivation systems.

Maximum weight gain occurred at temperatures that are known to be optimal for similar tropical / sub-tropical species, such as *Eucheuma denticulatum* and *Kappaphycus striatum* (Gerung and Ohno 1997). Expectedly, these optimal temperatures reflect those of their natural habitat (Lobban and Harrison 1997). In this study, an optimal temperature range can be defined, based on the statistical insignificance of growth / photosynthesis rates, and therefore we suggest that the range of optimum temperatures

**Table 2.** Photosynthesis versus irradiance ( $P-I$ ) parameters of *Betaphycus gelatinus*, *Eucheuma serra* and *Meristotheca papulosa* in this study

Parameters	<i>B. gelatinus</i>	<i>E. serra</i>	<i>M. papulosa</i>
$P_{\max}$ ( $\mu\text{g O}_2$ (mg chl-a) $^{-1}$ min $^{-1}$ )	135.0	65.0	52.4
$R_d$ ( $\mu\text{g O}_2$ (mg chl-a) $^{-1}$ min $^{-1}$ )	34.6	6.8	10.0
$\alpha$ (initial slope)	1.4	0.9	1.5
$I_k$ ( $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ )	94.9	69.4	35.4
$I_c$ ( $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ )	24.3	7.3	6.7

of *B. gelatinus* and *E. serra* were at least 24 and 28°C. Meanwhile, those of *M. papulosa* were thought to be 20 and 24°C. These optimal temperatures closely corresponded with those of their natural temperature. For example, although *B. gelatinus* can be found throughout a year at Ishigaki Is, Okinawa, meteorological data (Japan Meteorology Agency 2010) indicates that the mean monthly water temperature of Ishigaki Is. from January to December 2008 ranged from 23 to 30°C. Whereas, in the southern part of Kyushu I., *E. serra* and *M. papulosa* can be found only in the spring and summer season. At the time, the mean water temperature from May to July in Makurazaki, Kagoshima was in between 20 and 26°C, while in Ohtomari, Cape Sata of Kagoshima the temperatures ranged from 20 to 28°C from May to August (2008).

Our findings suggest that optimum temperatures of *B. gelatinus* and *E. serra* might be higher than *M. papulosa*, again reflecting the conditions of their natural environment and distribution, since *B. gelatinus* and *E. serra* can be frequently collected in relatively warmer environments than *M. papulosa*. This suggests that for tank cultivation, where water temperatures can exceed local water temperature due to phenomena such as solar heating, these more heat-tolerant species may be cultivated with relaxed temperature control. Indeed, Fig. 3 and 4 suggests that even at high temperatures, cultivation is possible. Hence, based on these findings cultivation of *B. gelatinus* may be conducted from throughout a year in Ishigaki Is., and from May to July for *M. papulosa* and *E. serra* at Makurazaki and Ohtomari of Cape Sata respectively under natural seawater temperatures.

Convergence of the nonlinear least squares regression of the hyperbolic tangent model was almost swift for all species (Fig. 4). Furthermore these were similar to that of *Kappaphycus alvarezii* reported by Schubert et al. (2006), although the saturating irradiances of the species we examined were somewhat lower. The biological significance of these differences should not be over-interpreted, given that handling and processing methods, as well as experimental protocol and environmental

history, can all lead to measurable changes in photosynthetic performance.

The model revealed that the photosynthetic parameters of *B. gelatinus* were, in general, highest among the three species. However, measured net photosynthesis at the initial slope (13, 24, 48 and 68  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ ) was relatively higher than those of estimated model. As the result of another calculation of initial slope by multiple linear regression coefficients,  $R_d$  (10.93  $\mu\text{g O}_2 (\text{mg chl-a})^{-1} \text{min}^{-1}$ ) and  $I_c$  (10.86  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$ ) were smaller than those of the hyperbolic tangent model. If so, the value of  $R_d$  and  $I_c$  might be similar among the three species. Therefore, a better model of the nonlinear least squares regression of the hyperbolic tangent might be required for future studies.

Nevertheless, in this study, *B. gelatinus* appeared to require higher levels of irradiance than *E. serra* and *M. papulosa* to saturate photosynthetic rates. We suggest that this is partly due to the irradiance conditions of their natural habitat, where *B. gelatinus* grow at around 2 m depth on the coral reef, *E. serra* can be found at 2–5 m, and *M. papulosa* grow subtidally from 3–20 m depth on the bedrock and boulders in wave-expose and as well as sheltered shores (Faye et al. 2005). Hence irradiance is reduced as water depth increases. Given the low values of irradiance needed to saturate photosynthesis, perhaps these species are shade-tolerant. For example, in one sub-tropical species of *Laurencia*, it was suggested that their peculiar distribution within a coral reef flat was partly related to photosynthetic performance (Nishihara et al. 2004a, 2004b). Indeed, in the species examined in this study, we often collected them in the “shadows” of rock. In the case of *B. gelatinus*, specimens that could be found on the top of the reef were prostrate, which can minimize exposure of the thallus surface to irradiance.

In conclusion, these results provide fundamental knowledge of temperature and irradiance conditions that are necessary to conduct land-based cultivation of these three important fisheries species. This study is unique in that it



provides information on both growth and photosynthetic processes, which are parameters that are often optimized in cultivation systems. Based on these results, we suggest that at the very least. The cultivation of *B. gelatinus* can be conducted throughout the year in Ishigaki Is. (Okinawa), while for *M. papulosa* and *E. serra* cultivation is possible from May to July in southern Kyushu Is. (Kagoshima and Miyazaki Prefectures). Through optimal selection of tank materials and cultivation setup, it may also be possible to cultivate these species year-round in hot-houses under both natural and artificial light, given the wide tolerance for temperature and generally low requirement for light.

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### References

- Anderson, R. (2005) *Algal Culturing Techniques*, Elsevier Academic Press, Burlington, USA, 596 pp.
- Dawes, C. J., A. C. Mathieson and D. P. Cheney (1974) Ecological studies of Floridian *Euclidean* (Rhodophyta, Gigartinales). I. Seasonal growth and reproduction. *Bull. Mar. Sci.*, **24**, 235-273.
- Faye, E. J., S. Shimada, S. Kawaguchi and M. Masuda (2005) Characterization of the edible red alga *Meristotheca papulosa* (Solieriaceae, Gigartinales) from Japan. *Phycol. Res.*, **53**, 234-245.
- Gerung, G. S. and M. Ohno (1997) Growth rates of *Euclidean denticulatum* (Burman) Collins et Harvey and *Kappaphycus striatum* (Schmitz) Doty under different conditions in warm waters of southern Japan. *J. Appl. Phycol.*, **9**, 413-415.
- Glenn, E. P. and M. Doty (1990) Growth of the seaweeds *Kappaphycus alvarezii*, *K. striatum* and *Euclidean denticulatum* as affected by environment in Hawaii. *Aquaculture*, **84**, 245-255.
- Henley, W. J. (1993) Measurement and interpretation of photosynthetic light-response curves in algae in the context of photo inhibition and diel changes. *J. Phycol.*, **29**, 729-739.
- Hung, L. D., K. Hori, H. Q. Nang, T. Kha and L. T. Hoa (2009) Seasonal changes in growth rate, carrageenan yield and lectin content in the red alga *Kappaphycus alvarezii* cultivated in Camranh Bay, Vietnam. *J. Appl. Phycol.*, **21**, 265-272.
- Japan Meteorology Agency (2010) [http://www.data.kishou.go.jp/kaiyou/db/nagasaki/db\\_nagasaki.html](http://www.data.kishou.go.jp/kaiyou/db/nagasaki/db_nagasaki.html)
- Jassby, A. D. and T. Platt (1976) Mathematical formulation of the relationship between photosynthesis and light for phytoplankton. *Limnol. Oceanogr.*, **21**, 540-547.
- Kawakubo, A., H. Makino, J. Ohnishi, H. Hirohara and K. Hori (1997) The marine red alga *Euclidean serra* J. Agardh, a high yielding source of two isolectins. *J. Appl. Phycol.*, **9**, 331-338.
- Kawakubo, A., H. Makino, J. Ohnishi, H. Hirohara and K. Hori (1999) Occurrence of highly yielded lectins homologous within the genus *Euclidean*. *J. Appl. Phycol.*, **11**, 149-156.
- Lobban, C. S. and P. J. Harrison (1997) *Seaweeds Ecology and Physiology*, Cambridge University Press, Cambridge, UK, 384 pp.
- Maegawa, M., M. Kunieda and W. Kida (1993). The influence of ultraviolet radiation on the photosynthetic activity of several red algae from different depth. *Jpn. J. Phycol.*, **41**, 207-214.
- Muraoka, D., H. Yamamoto, H. Yasui and R. Terada (1998) Formation of wound tissue of *Gracilaria chorda* Holmes (Gracilariaceae) in culture. *Bull. Fac. of Fish., Hokkaido Univ.*, **49**, 31-39.
- Murase, N., M. Maegawa and W. Kida (1989) Photosynthetic characteristics of several species of Rhodophyceae from different depth in the coastal area of Shima Peninsula, central Japan. *Jpn. J. Phycol.*, **37**, 213-220.
- Nishihara, G. N., R. Terada and T. Noro (2004a) Photosynthesis and growth rates of *Laurencia brongniartii* J. Agardh (Rhodophyta, Ceramiales) in preparation for cultivation. *J. Appl. Phycol.*, **16**, 303-308.
- Nishihara, G. N., R. Terada, Y. Mori and T. Noro (2004b) Habitat characteristics and seasonal changes of *Laurencia brongniartii* (Ceramiales, Rhodophyta) in Kagoshima, Southern Japan. *Phycol. Res.*, **52**, 30-37.
- Nishihara, G. N., Y. Mori, R. Terada and T. Noro (2004c) A simplified method to isolate and cultivate, *Laurencia brongniartii* J. Agardh (Rhodophyta, Ceramiales) from Kagoshima, Japan. *Suisanzoshoku*, **52**, 1-10.
- Nishihara, G. N. and R. Terada (2010) Spatial variations in nutrient supply to the red algae *Euclidean serra* (J. Agardh) J. Agardh. *Phycol. Res.*, **58**, 29-34.
- Ohno, M., M. Yano, M. Hiraoka, N. Oka and M. Taniguchi (2002) Tank culture of *Euclidean serra* and *Meristotheca papulosa* using with deep sea water. *Bull. Mar. Sci. Fish., Kochi Univ.*, **20**, 35-40 (in Japanese).
- Ohno, M. (2004) Edible local seaweeds. In "Biology and



- Technology of Economic Seaweeds*" (ed. by M. Ohno), Uchida Rokakuho Publishing Co. Ltd., Tokyo, pp. 283-296.
- Parker, H. S. (1974) The culture of the red algal genus *Euclima* in the Philippines. *Aquaculture*, **3**, 425-439.
- Paula, E. J., C. Erbert and R. T. L. Pereira (2001) Growth rate of the carrageenophyte *Kappaphycus alvarezii* (Rhodophyta, Gigartinales) *in vitro*. *Phycol. Res.*, **49**, 155-161.
- Paula, E. J., R. T. L. Pereira and M. Ohno (2002) Growth rate of the carrageenophyte *Kappaphycus alvarezii* (Rhodophyta, Gigartinales) introduced in subtropical waters of São Paulo State, Brazil. *Phycol. Res.*, **50**, 1-9.
- Porra, R. J., W. A. Thompson and P. E. Kriedemann (1989) Determination of accurate extinction coefficients and simultaneous for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. *Biochim. Biophys. Acta Bioenerg.*, **975**, 384-394.
- Santos, G. A. (1989) Carrageenans of species of *Euclima* J. Agardh and *Kappaphycus* Doty (Solieriaceae, Rhodophyta). *Aquat. Bot.*, **36**, 55-67.
- Schubert, H., M. Anderson and P. Snoeijs (2006) Relationship between photosynthesis and non-photochemical quenching of chlorophyll fluorescence in two red algae with different carotenoid compositions. *Mar. Biol.*, **149**, 1003-1013.
- Serisawa, Y., Y. Yokohama, Y. Aruga and J. Tanaka (2001) Photosynthesis and respiration in bladelet of *Ecklonia cava* Kjellman (Laminariales, Phaeophyta) in two localities with different temperature conditions. *Phycological Research*, **49**, 1-11.
- Shinmura, I. (2000) *Meristotheca*. In "History of Fisheries Technology in Kagoshima Prefecture" (ed. by Kagoshima Prefecture), Kagoshima Prefecture, Kagoshima, pp. 427-431 (in Japanese).
- Shinmura, I. and T. Tanaka (2008) Useful seaweeds of Kagoshima III. Rhodophyta. *Jpn. J. Phycol.*, **56**, 123-128 (in Japanese).
- Terada, R. and H. Yamamoto (2000) A taxonomic study on two Japanese species of *Gracilaria*: *G. shimodensis* sp. nov. and *G. blodgettii* (Gracilariales, Rhodophyta). *Phycological Research* **48**, 189-198.
- Trono, G. C. Jr. (1993) *Euclima* and *Kappaphycus*: Taxonomy and cultivation. In "Seaweed Cultivation and Marine Ranching" (ed. by M. Ohno and A. T. Critchley), Japan International Cooperation Agency (JICA), Yokosuka, pp. 75-88.
- Yokohama, Y. (1973) Photosynthetic properties of marine benthic red algae from different depth in the coastal area. *Bull. Jap. Soc. Phycol.*, **21**, 119-124 (in Japanese).
- Yoshida, T. (1998) *Marine Algae of Japan*, Uchida Rokakuho Publishing Co., Ltd. Tokyo, 1,222 pp.
- Yoshida, T. and K. Yoshinaga (2010) Checklist of marine algae of Japan (revised in 2010). *Jpn. J. Phycol.*, **58**, 69-122.

## 食用海藻3種、カタメンキリンサイ、トゲキリンサイ、トサカノリ（紅色植物門ミリン科）の室内培養による生長及び光合成

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カタメンキリンサイ、トゲキリンサイ、トサカノリ（紅色植物門ミリン科）は、熱帯・亜熱帯域に生育する有用海藻であり、日本では南西諸島や九州でよく見られる。本研究では、これら3種の養殖技術を確立する上で必要な至適光・温度条件を検討することを目的とした。温度（16, 20, 24, 28, 32℃）が生長や光合成に与える影響については、培養による生長試験と溶存酸素計を用いた光合成試験の2つの実験で行った。また、水温24℃、光量0から536  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  の条件で光合成速度を測定し、光合成-温度曲線を作成した。光量90  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  における最適生長率はカタメンキリンサイとトゲキリンサイで24℃と28℃、トサカノリで20℃と24℃の範囲だった。最大光合成速度はカタメンキリンサイで135.0、トゲキリンサイで65.0、トサカノリで52.4  $\mu\text{g O}_2 \text{ (mg chl-a)}^{-1} \text{ min}^{-1}$  であり、それぞれ94.9, 69.4, 35.4  $\mu\text{mol photon m}^{-2} \text{s}^{-1}$  以上で飽和した。これらの結果は各種の生育水深と日本南部における分布と密接に関連しており、各種の養殖可能時期について考察を行った。