Physiological Study of Carrageenophytes (*Betaphycus gelatinus, Eucheuma serra, E. denticulatum, Meristhotheca coacta, M. papulosa* and *Kappaphycus* sp., Rhodophyta) from Japan and Indonesia

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LIDEMAN

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Dedicated to:

My beloved children, Naurah, Nabil and their mother for the love, patience and inspiration.

My mother who has passed away during I stayed in Japan, my father, my brothers and my sisters for their attention, support and love.

Kagoshima,
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ABTRACT

Some species of Solieriaceae (Rhodophyta, Red Algae) *i.e*. *Betaphycus gelatinus*, *Eucheuma serra*, *E. denticulatum*, *Meristotheca coacta*, *M. papulosa* and *Kappaphycus* sp. (Sumba strain) were used in this study in order to examine their physiological activities based on their growth and photosynthetic performance affected by environmental factors especially temperature and light. This study was conducted base on a necessary to cultivate of these seaweeds because of their *carrageenan* content, intense harvesting in their habitat and the ocean global warming issue. Japan is one of the largest *carrageenan* importing countries in the world and the warm seawater area in southern part of Japan could be used in order to cultivate these *carrageenophytes* (macroalgae as a source of *carrageenan*) and to prevent them from over harvesting as edible seaweeds. Furthermore, increasing temperatures as a result of global warming may lead these macroalgae to change in spatial distribution in the future. However, there was a lack of basic information on how to cultivate these macroalgae, especially Japanese species.

An *in vitro* growth and photosynthesis study of *B. gelatinus*, *E. serra* and *M.*

papulosa affected by temperatures and light were conducted. The growth rate of *B. gelatinus* and *E. serra* were optimum at temperature 24 to 28 °C and for *M. papulosa*, it was at temperature of 22 - 24 °C. At temperature 24 °C, *B. gelatinus*, *E. serra* and *M. papulosa* reached their saturating irradiance at 94.9, 69.4 and 35.4 μmol photons m^{-2} s^{-1}, respectively. The cultivation of *B. gelatinus* can be conducted throughout a year in Ishigaki Is. (Okinawa), while for *M. papulosa* and *E. serra* cultivation is possible from May to August in southern part of Kyushu Is. (Kagoshima and Miyazaki Prefectures).

The author used the pulse amplitude modulated (PAM)-chlorophyll fluorometry (Imaging-PAM) in order to examine photosynthetic parameters of *M. coacta* and *M. papulsa* affected by temperatures and light. This equipment has been used for some seagrasses as well as terrestrial plants and can be applied quickly and efficiently in analyzing of photosynthetic response from the intact plants. Both species required temperatures ranging from 18 to 28 °C and the saturating photosynthetic active radiation (PAR_{sat}) increased with increasing temperatures. I provided equations of relation among the photosynthetic parameters and temperatures which should be useful to design and manage the program of mariculture and tank cultivation system. I suggest that both of the

species can be successfully cultivated from April to August in Kyusyu Island (Kumamoto, Kagoshima and Miyazaki Prefecture).

Finally, the photosynthetic performances of tropical carrageenophyte species affected by temperatures and light were also investigated in Chapter 4. Cultured *E. denticulatum* and *Kappaphycus* sp. (Sumba strain) were collected at farming area in Funaga, South Sulawesi (Indonesia). PAM-chlorophyll fluorometry (Diving-PAM) was used to examine photosynthetic parameters. These results suggest that the optimal temperatures of these two species is most likely with the range of 22 to 32 °C, and corresponds well to surface temperature recorded in Indonesian ocean. Similar with result in chapter 3, PAR_{sat} value of *E. denticulatum* and *Kappaphycus* sp. measured in this study increased with increasing of water temperature.

要　旨

紅藻カタメンキリンサイ (*Betaphycus gelatinus)*、トゲキリンサイ (*Eucheuma serra)*、キリンサイ (*E. denticulatum)*、キクトサカ (*Meristotheca coacta*)、トサカノリ（*M. papulosa*）ならびに *Kappaphycus* sp. (Sumba strain)（ミリン科）の生長と光合成活性に及ぼす温度と光量の影響を明らかにした。

まず、カタメンキリンサイ、トゲキリンサイとトサカノリの生長と光合成活性に与える水温と光度の影響を室内実験により明らかにした。それによればカタメンキリンサイとトゲキリンサイの生長速度は 24～28℃で最大となったのに対し、トサカノリは 22～ 24℃で最大となった。また、24℃における飽和光度を酸素電極を用いて測定したところ、各々 94.9、 69.4 と 35.4 μ mol photons m^{-2} s^{-1}の値を示した。ちなみに、カタメンキリンサイは沖縄県石垣島においては周年にわたり生育するが、九州南部に生育するトサカノリやトゲキリンサイは５～７月に最大となる種である。

次に、 PAM 光合成測定器 (Imaging-PAM)を用いてキクトサカとトサカノリの光合成に及ぼす温度と光度の影響を明らかにした。それによれば 18～28℃における両種の光合成有効放射(PAR_{sat}) は温度の上昇にともなって増加した。この結果は、本種を培養する上で考慮されるべきであり、九州南部においては５～8 月が生長に適することが証明された。

さらに、インドネシア南スラウェシ産のキリンサイと *Kappaphycus* sp. (Sumba 株) の光合成活性を水中用 Diving-PAM によって測定した。それによれば、22-32℃の温度範囲においては温度上昇にともない PAR_{sat} も増加することが分かったが、これはインドネシアの水温域での生長を裏付けるものであった。

本研究はこれらのカラギーナン含有藻類を養殖する時に必要な知見を得るのみならず、地球温暖化にともなう地理的分布を予想するために必要とされよう。

CHAPTER 1: GENERAL INTRODUCTION

Specimens used in this study are commercially important product because of their *carrageenan* content. The *carrageenan* derived from these red algae is used in many industries such as industries of food, cosmetic, textile and pharmacy as a firming agent, emulsifier, coagulating agent, and filling material (Waaland *et al*., 1981). Crude extract of various algal polysaccharides (agar, carrageenan, algin and related colloids) have been used since the middle of 17^{th} century (Chapman, 1970) in China, Japan and Indonesia, while the carrageenan was produced as a commercial extraction product in 1930's in England and it was used as chocolate stabilizer (Waaland *et al*, 1981). Traditional raw material for carrageenan was extracted from *Chondrus crispus* (Irish moss) of red algae (Tseng *et al*., 1981) and this *Chondrus crispus* was commercially harvest first time in Carragheenan, Irland (Waaland *et al*., 1981)

Mariculture of genera *Kappaphycus* and *Eucheuma* supplies most of the *carrageenan* and carrageenan-like products (Dawes *et al*., 1994). *Eucheuma gelatinae* (as *Betaphycus gelatinus*) has been commercially cultivated in China since 1960 (Waaland *et al*., 1981). Commercial cultivation of *Kappaphycus alvarezii* was developed in the Philippines during the latter half of the 1960's using local varieties selected from the wild (Parker, 1974).

In Southeast Asia, genus *Eucheuma* and *Kappapycus* are the prominent commodity that cultivated largely particularly in Indonesia and Philippine (Bixler and Porse, 2010). Indonesia has become the largest exporter of *Eucheuma* raw materials, supplying the foreign demand for *iota*-carrageenan and increasing demand of the Asian food market (Adnan and Porse, 1987; Luxton, 1993). It has also been reported that cultivation of *Kappaphycus* has been successful in China (Wu *et al*., 1989), Madagascar (Mollion and Braud, 1993), and Vietnam (Ohno *et al*., 1996).

These relative successes are apparently due to similar environmental conditions between the recipient transplanted sites and that from where the *Eucheuma* was originally collected (Ohno *et al*., 1994). In fact, Japan has been one of the largest *carrageenan* importing countries in the world (Ohno *et al*., 1994), the warm seawater area which is in southern part of Japan could be used in order to cultivate carrageenophytes as a source of *carrageenan*. In other hand, some red algae species growing in southern part of Japan are the edible seaweeds harvested by fishermen for soup, salad and "*canten*" (a food for substituting agar). Notably, *E. serra* and *M. papulosa* are mainly harvested in southern Kyushu Is. (Kagoshima and Miyazaki Prefecture), and *B. gelatinus* is harvested in Ishigaki and Miyako Is. of Okinawa Prefecture. They are indispensable to 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).

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±0.26 °C over a period of 1900 to 2010 (JMA, 2011b). Changing environmental conditions can be expected to influence the harvest of these species.

Study on cultivating of *K. alvarezii* as source of *carrageenan* in Japan was conducted by Ohno *et al*. (1994) and reported that the species had daily growth rate up

to 8% in autumn while die-back occurred in winter due to low temperature (15 °C). In order to solve the low temperature problem, using a carragenophyte from Japan become necessary. Species of genera *Betaphycus*, *Eucheuma* and *Meristotheca* grow well along the shores of southern part of Japan (Yoshida, 1998), with seven species recognized for the region (Yoshida and Yoshinaga, 2010). Three genera, *Eucheuma, 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). Genus *Meristotheca* could be also a candidate for cultivating in Japan because they grow naturally in Japan and *carrageenan* has been isolated from some species of *Meristotheca*, such as *M. papulosa* (Usov *et al*., 2001) and *M. senegalensis* (Fostier *et al*., 1992).

Some strains of *Kappaphycus alvarezii* are cultured in Indonesia such as *Sakol, Tambalang, cottonii* and *Sumba*. Sumba strain (*Kappaphycus* sp.) is predicted come from Sumba Island in Indonesia, while Sakol and Tambalang strain are come from the Philippines. Although, *Sumba* strain is prominent commodity cultured in Indonesia, study on their physiological aspects especially photosynthesis is very restricted. Species of *K. alvarezii* is mostly use 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). Some 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.

Since the culvitvation of some carrageenophytes (*Betaphycus gelatinus*, *Eucheuma serra*, *E. spinosum*, *Meristotheca papulosa*, *M. papulosa* and *Kappaphycus* sp.) are very importance, however the basic information of some factors for developing aquaculture system is necessary. Therefore, this study aims are to provide basic information about environmental factors especially temperature and light affecting physiological aspect of some carrageenophytes. The basic information of this study will lead to development their aquaculture system.

In this study, I examine the possibility on cultivating of some carrageenphytes (Red Algae) growing in Japan (*B. gelatinus*, *E. serra, M. coacta* and *M. papulosa*) based on their *in vitro* growth and photosynthetic performance. I show how the environmental factors especially temperature and light affecting the photosynthetic activity in order to develop their aquaculture system. Cultured tropical carrageenophytes (Red Algae) in

Indonesia (*E. denticulatum* and *Kappaphycus* sp. (Sumba strain)) were also used to examine their photosynthetic performance affected by temperature and light in chapter 4. Photosynthetic activity was examined by using dissolved oxygen evolution in chapter 2. And pulse amplitude modulated (PAM)-chlorophyll fluorometry (Imaging-PAM and Diving-PAM) was used as a quick and efficient technique, first developed to study photosynthesis in intact plants (chapter 3 and 4).

CHAPTER 2: GROWTH AND PHOTOSYNTHESIS OF *Betaphycus gelatinus*, *Eucheuma serra* AND *Meristotheca papulosa*

INTRODUCTION

Temperate red algal family, Solieriaceae (Gigartinales), includes many economically important species, of which three genera, *Eucheuma*, *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 also grow well 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 there are three species grow in southern part of Japan (Yoshida and Yoshinaga, 2010).

Fig. 2.1. Rhodophyta in their natural environment used in this experiment. a, *Betaphycus gelatinus*; b, *Eucheuma serra*; c, *Meristotheca papulosa*.

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. 2.1a), *Euchemua serra* (J. Agardh) J. Agardh (Fig. 2.1b) and *Meristotheca papulosa* (Montagne) J. Agardh (Fig. 2.1c) 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 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, unpublished data) 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 innovate 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 *Euchuema sensu lato* (including *Kappaphycus* and *Betaphycus*) or *Meristotheca* cultivation systems has been established in Japan. Although a trial experiment of *M. papulosa* aquaculture has started by the Government of Ehime Prefecture (unpublished), but its system is still incomplete including optimum temperature and irradiance conditions. I suggest that optimum temperature and light conditions, and growth characteristics must be elucidated so we can develop an advance cultivation technology, strategy, and conserve natural populations.

In this study, I 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. 2.1.a) were collected from of Yoshiwara, Ishigaki Island, Okinawa Prefecture (24˚27’25”N, 124˚09’20”E; Depth: 2 m) on December 26th, 2007. *Eucheuma serra* (Fig. 2.1.b) and *Meristotheca papulosa* (Fig. 2.1c) were collected in Kagoshima Prefecture at Makurazaki (31˚15’58”N, 130˚17’24”E; Depth: 10 m) on 9th May, 2008 and at Ohtomari near Cape Sata (31˚01’14N, 130˚41’09”E; Depth: 5 m) on 1st June, 2008, respectively. Each sample was collected by SCUBA and skin diving by authors or local fishermen. 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^3) containing seawater of salinity at 33 PSU, pH of 8.0, 90 μ mol photons m^{-2} s^{-1} and 20°C under 14:10 light: dark cycle. Temperature of 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 specimen 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 GeO_2 (10 mg GeO_2/1 *l* PES), 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 μmol photons m^{-2} 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°C at the same fluorescent lamp with same light irradiance (90 μmol photons m^{-2} s^{-1}) to examine the effects of temperature on the growth of *B. gelatinus*, *E. serra* and *M. papulosa*. 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, % day^{-1}) were calculated over the exponential growth phase using the following equations:

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

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

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°C with four replicates were examined at an irradiance of 248 μ mol photons $m^{-2}s^{-1}$ that was higher than saturation irradiance (I_k) derived from below mentioned photosynthetic versus irradiance (*P-I*) curve, 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 temperature controlled water bath (Coolnit CL-80F, Taitec, Inc., Tokyo). Photosynthetic rates were determined by measuring the dissolved oxygen concentration (mg O_2/ *l*) every 5 minutes for 30 minutes after pre-incubation for 30 minutes acclimatization at each experimental condition. Dissolve 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 (mg_{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 μ mol photons m^{-2} s^{-1} with four replicates at 24°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:

$$P_{net} = (P_{max} \times \tanh [\alpha / P_{max} \times I]) + R_d \qquad (3)$$

where, P_{net} was the net O_2 production rate, P_{max} was the maximum O_2 production rate, α was the initial slope of the photosynthesis versus irradiance curve, I was the incident irradiance, and R_d was the dark respiration rate (Jassby and Platt, 1976; Henley, 1993).

Saturation irradiance (I_k) was calculated as P_{max} / α and compensation irradiance (I_c) was R_d / α (Schubert *et al*., 2006).

Measurement of chlorophyll-a

Samples were extracted under refrigeration by using 10 *ml* of 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:

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

where $Abs_{663.8}$ was the absorbance at 663.8 nm, $Abs_{646.8}$ was the absorbance at 646.8 nm and Abs_{700} was the absorbance at 700 nm.

Statistical analysis

A one-way ANOVA ($P < 0.05$) was used to examine how the temperature affected the growth rate and photosynthetic rate by using the software Supernova. The Duncan new multiple range 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. 2.2). Initial weight of *B. gelatinus* was

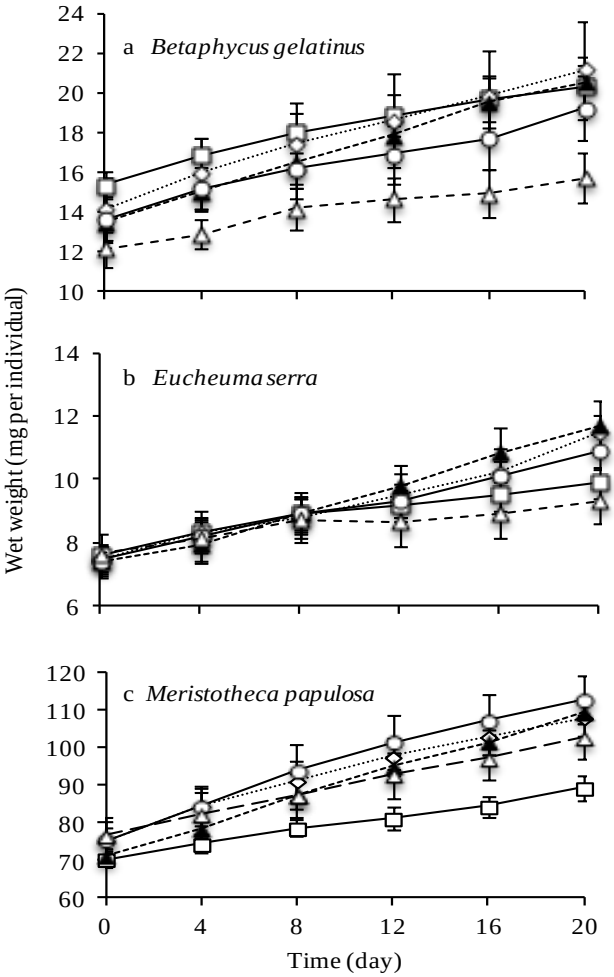

Fig. 2.2. *In vitro* growth of *Betaphycus gelatinus* (a), *Eucheuma serra* (b) and *Meristotheca papulosa* (c) at the five different temperature levels in 20-days cultures (△, 16˚C; ○, 20˚C; ▲, 24˚C; ◇, 28˚C; □, 32˚C). Bars show the standard deviation, n = 5.

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 2.1). 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*

Table 2.1. Weight gain (%) of *Betaphycus gelatinus*, *Eucheuma serra* and *Meristotheca papulosa* at the five different temperature levels after 20-days *in vitro* cultures. Errors show the standard deviation (n=5)

	16˚C	20˚C	24˚C	28˚C	32˚C
Betaphycus gelatinus	29.4 ± 3.7	41.3 ± 3.2	52.9 ± 3.7	49.4 ± 3.7	32.4 ± 3.6
Eucheuma serra	22.3 ± 2.7	46.0 ± 4.0	57.6 ± 4.3	53.5 ± 4.5	30.2 ± 3.1
Meristotheca papulosa	34.1 ± 2.6	49.9 ± 4.9	53.2 ± 3.9	43.2 ± 2.8	26.9 ± 2.9

occurred at 32˚C with rate of 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^{-1} at 24°C followed by 2.0% day^{-1} at 28°C (Fig. 2.3). However, these RGR were not significantly different (P< 0.05) each other. The RGR at 20˚C and 32°C with value of 1.7 and 1.4% day^{-1}, respectively was

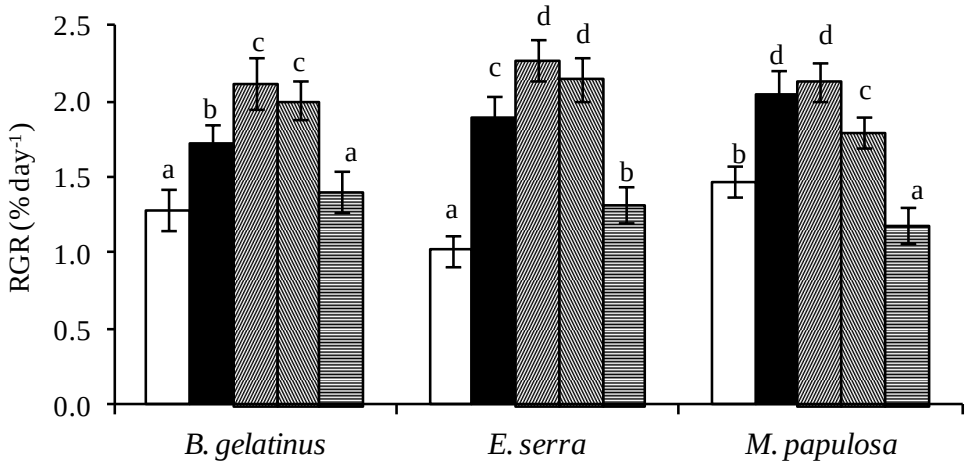

Fig. 2.3. Relative growth rate (RGR) of *Betaphycus gelatinus*, *Eucheuma serra,* and *Meristotheca papulosa* at the differrent temperature levels in 20-days cultures (□, 16˚C; ■, 20˚C; ▨, 24˚C; ▧, 28˚C; ▤, 32˚C). Bars show the standard deviation, n=5. Means with different alphabet (a, b, c, d) are significantly different (P<0.05).

significantly lower compared to 24°C and 28°C. The lowest RGR of *B. gelatinus* was 1.3% day^{-1} at 16˚C and did not significantly differ with RGR at 30°C. The highest RGR of *E. serra* was 2.3% day^{-1} at 24°C, but was not significantly different with RGR at 28°C with rate of 2.2% day^{-1}. Moreover, the RGR significantly decreased (P < 0.05) to 1.9% day^{-1} at 20°C, followed by 1.3 and 1.0% day^{-1} at 32°C and 16°C, respectively. In case of *M. papulosa*, maximum RGR was 2.1% day^{-1} occurred at 24°C, and it was not

significantly different ($P < 0.05$) with RGR at 20°C with value 2.0% day^{-1}. 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^{-1}, respectively.

Chlorophyll-a content

The average of chlorophyll-a contents of *B. gelatinus, E. serra and M. papulosa* were 29.5, 110.6 and 108.5 $\mu g/g_{ww}$ respectively (Table 2.2). The chlorophyll-a content of *B. gelatinus* was relatively lower compared to the chlorophyll-a content of *M. papulosa and E. serra.*

Table 2.2. Chlorophyll-a content of *B. gelatinus, E. serra and M. papulosa*

Species	Replicates	Chlorophyll-a ($\mu gchlo\text{-}a/g_{ww} \pm$ s.d.)
B. gelatinus	32	29.5 ± 8.8
E. serra	12	110.6 ± 7.7
M. papulosa	24	108.5 ± 13.6

Note: s.d. was standard deviation; g_{ww}: gram wet weight

Effect of temperature on the photosynthetic rate

Photosynthetic rates of three macroalgae based on chlorophyll-a are presented in Fig. 2.4. 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

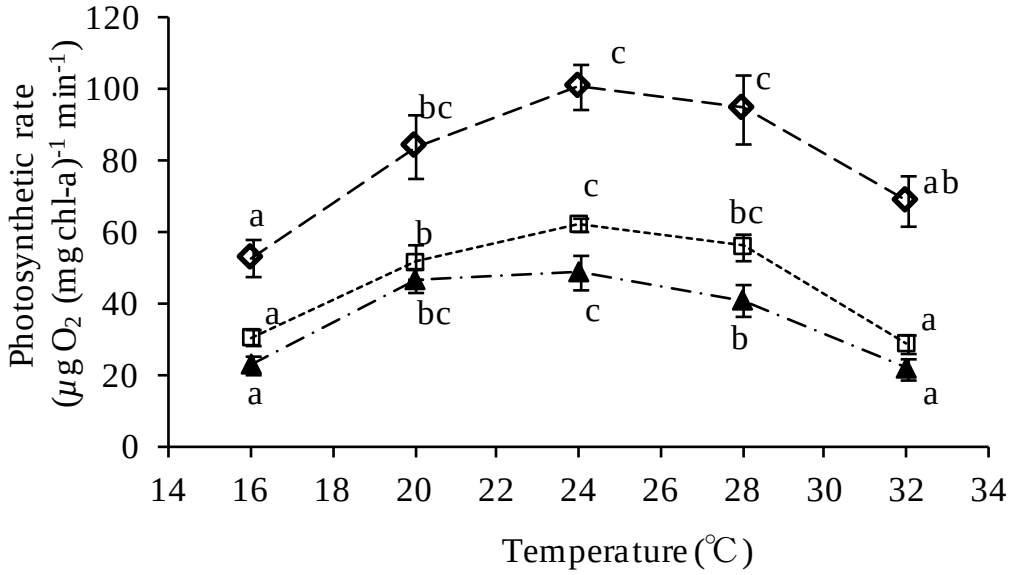

Fig. 2.4. Photosynthesis versus temperature (*P-T*) curve of *Betaphycus gelatinus* (◇), *Eucheuma serra* (□) and *Meristotheca papulosa* (▲) at the different temperature. Bars show the standard deviation, n = 4. Means with different alphabet (a, b, c) are significantly different (P<0.05).

highest photosynthetic rates were shown 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 μ g O_2 (mg chl-a)$^{-1}$ min^{-1} at 24°C and was not significantly different with photosynthetic rate at 20 and 28°C. Furthermore, the lowest photosynthetic rate was 52.7 μ g O_2 (mg chl-a)$^{-1}$ min^{-1} found at 16°C (Fig. 2.4).

As for *M. papulosa*, increasing in temperature from 16°C to 24°C increased the photosynthetic rate from 22.9 to 48.8 μ g O_2 (mg chl-a)$^{-1}$ min^{-1} and dropped to 21.7 μ g O_2 (mg chl-a)$^{-1}$ min^{-1} at 32°C. Maximum photosynthetic rate at 24°C was not

significantly ($P < 0.05$) different with rate at 20°C (Fig. 2.4).

Similarly, the highest photosynthetic rate of *E. serra* was also found at 24°C with rates of 62. 2 μ g O_2 (mg chl-a)$^{-1}$ min^{-1}, followed by 56.0, 51.5 and 30.4 μ g O_2 (mg chl-a)$^{-1}$ min^{-1} at 28, 20 and 16°C, respectively. The lowest photosynthetic rate was 28.8 μ 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 photosynthetic rate at 28°C ($P < 0.05$), but significantly different with other treatments ($P < 0.05$) (Fig. 2.4).

Effect of irradiance on the photosynthetic rate

Photosynthetic rates could be modeled with the hyperbolic tangent form of the *P-I* equation (Fig. 2.5). Respiration rates (R_d) of *B. gelatinus, E. serra* and *M. papulosa* was 34.6, 6.8 and 10.0μ g O_2 (mg chl-a)$^{-1}$ min^{-1}, respectively. Photosynthetic rates increased sharply from 13 to 168 μ mol photons m^{-2} s^{-1} and then increased slowly until 248 μ mol photons m^{-2} s^{-1}. Maximum photosynthetic rates of *B. gelatinus, E. serra* and *M. papulosa* was 100.8, 62.2 and 48.8μ g O_2 (mg chl-a)$^{-1}$ min^{-1}, respectively, and all photosynthetic rates reached their maximal values by 248 μ mol photons m^{-2} s^{-1}.

Regarding the parameters of the *P-I* model, P_{max} for *B. gelatinus, E. serra* and *M. papulosa* was 135.0, 65.0 and 52.4 μ g O_2 (mg chl-a)$^{-1}$ min^{-1}, respectively; saturating

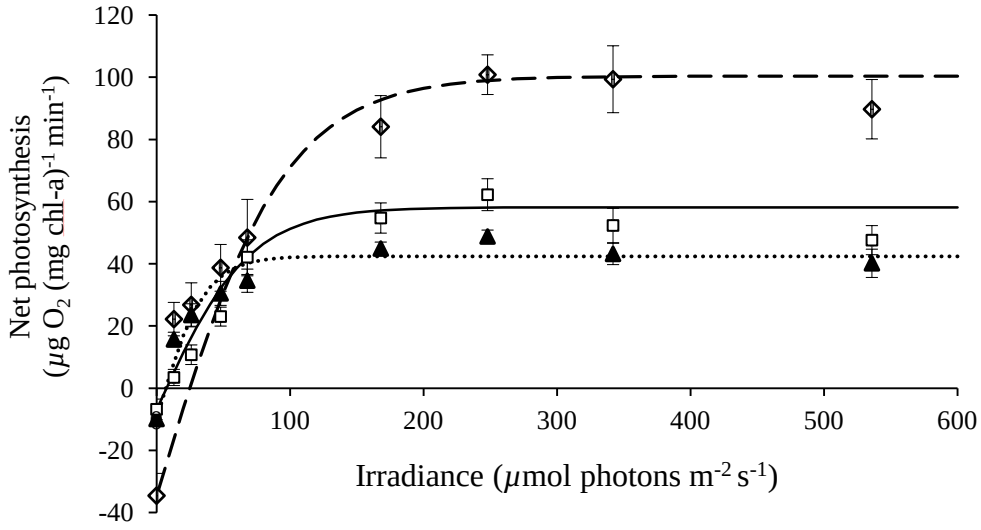

Fig. 2.5. Photosynthesis versus Irradiance (*P-I*) curve model of *Betaphycus gelatinus* (◇), *Eucheuma serra* (□) and *Meristotheca papulosa* (▲). Bars show the standard deviation, n = 4.

irradiance (I_k) was occurred at 94.9, 69.4 and 35.4 μmol photons m^{-2} s^{-1}, respectively and compensation irradiance (I_c) occurred at 24.3, 7.3 and 6.7 μ mol photons m^{-2} s^{-1}, respectively (Table 2.3).

Table 2.3. Photosynthesis versus irradiance (P-I) parameters of *Betaphycus gelatinus*, *Eucheuma serra* and *Meristotheca papulosa* in this study

Parameters	*B. gelatinus*	*E. serra*	*M. papulosa*
P_{max} (μgO_2(mg chl-a)$^{-1}$ min^{-1})	135.0	65.0	52.4
R_d (μgO_2(mg chl-a)$^{-1}$ min^{-1})	34.6	6.8	10.0
α (initial slope)	1.4	0.9	1.5
I_k (μmol photons m^{-2} s^{-1})	94.9	69.4	35.4
I_c (μmol photons m^{-2} s^{-1})	24.3	7.3	6.7

DISCUSSION

The successful cultivation of macrophytes, such as the three species I 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 of optimum temperature and irradiance parameters might be contributed to consider the suitable mariculture (depth and culture period) and tank culture condition.

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, on the basis of the range that can be regarded to be statistically indifferent with a rate at the highest temperature, I suggest that the range of optimum temperatures 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 Is., *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. 2.4. 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. 2.5). Furthermore these were similar to that of *Kappaphycus alvarezii* reported by Schubert *et al*. (2006), although the saturating irradiances of the species I 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 μ mol photons m^{-2} 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 μ g O_2 (mg chl-a)$^{-1}$ min^{-1}) and I_c (10.86 μ mol photons m^{-2} s^{-1}) were smaller than those of the hyperbolic tangent model. If so, the value of *R*d and *I*c might be similar during three species. To conclude them, application of much 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. I 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 with 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 both growth and photosynthetic processes, which are parameters that are often optimized in

cultivation systems. Based on these results, I suggest that cultivation of *B. gelatinus* can be conducted throughout a year in Ishigaki Is. (Okinawa), while for *M. papulosa* and *E. serra* cultivation is possible from May to August in southern part of 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.

CHAPTER 3: EFFECT OF TEMPERATURE AND LIGHT ON THE PHOTOSYNTHESYS OF *Meristhotheca coacta* AND *Meristotheca papulosa* FROM JAPAN

INTRODUCTION

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). Indeed, in coastal ecosystems of Japan, three species of *Meristotheca*, *M. coacta* Okamura, *M. imbricata* Faye *et* Masuda and *M. papulosa* (Montagne) J. Agardh, can be observed (Yoshida and Yoshinaga, 2010), and which *M. imbricate* is a newly described endemic species to this region (Faye *et al*., 2008). The other two species can also be found in

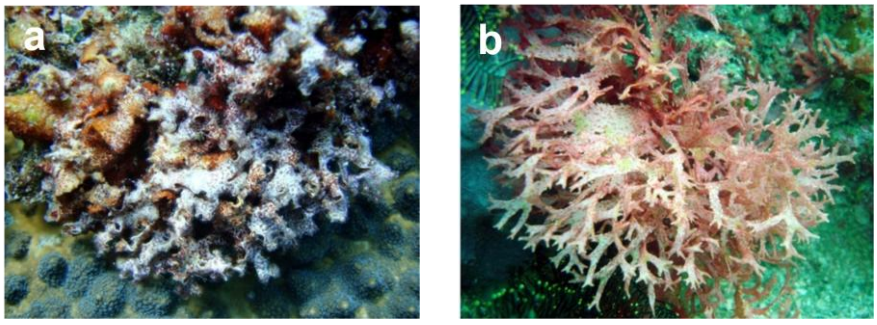

Fig. 3.1. *Meristotheca coacta* (a) and *M. papulosa* (b) in their natural habitat of Cape Sata and Ushibuka (Amakusa-Shimojima Is.), respectively.

other parts of Asia; *M. coacta* (Fig. 3.1.a) can be found in Korea (Lee and Kang, 2001), Taiwan (Huang, 2000) and the Philippines (Kraft *et al*., 1999) and *M. papulosa* (Fig. 3.1.b) has been reported from along the coast of Indo-Pacific countries (Faye *et al*.,

2005). In this study, I focus on *Meristotheca* because it is an important fisheries resource to many countries of this region. For instance, *M. papulosa* is a popular edible seaweed and is used as the ingredient for salads in Japan, especially in the southern 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. papulosa* (Usov *et al*., 2001) and *M. senegalensis* (Fostier *et al*., 1992), and can be considered a viable source of this valuable bioproduct.

Nevertheless, through intense harvesting and other anthropogenic activity, concern has been 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 447,800 kg wet weight of biomass with a value of 331.7 million yen was harvested in 2000, and 302,860 kg wet weight with a value of 195.8 million yen was harvested in 2006. As I have previously noted, *M. imbricata* also occurs in this region, although they appear to be restricted to

parts of Southern Kyushu. In contrast, *M. coacta* is 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 a change of distribution 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 ± 0.26 °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 electro-chemical techniques (Yokohama, 1973; Murase *et al*., 1989; Maegawa *et al*., 1993). 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, I apply a quick and efficient technique, first developed to study photosynthesis in intact plants (pulse amplitude modulated (PAM)-chlorophyll fluorometry; Kuster *et al*., 2007; Aldea *et al*., 2006; Ralph *et al*., 2006). 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

M. 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 May 15, 2010 (Fig. 3.2.). *M. coacta* and *M. papulosa* were collected at water depth ranging from 1 – 5 m.

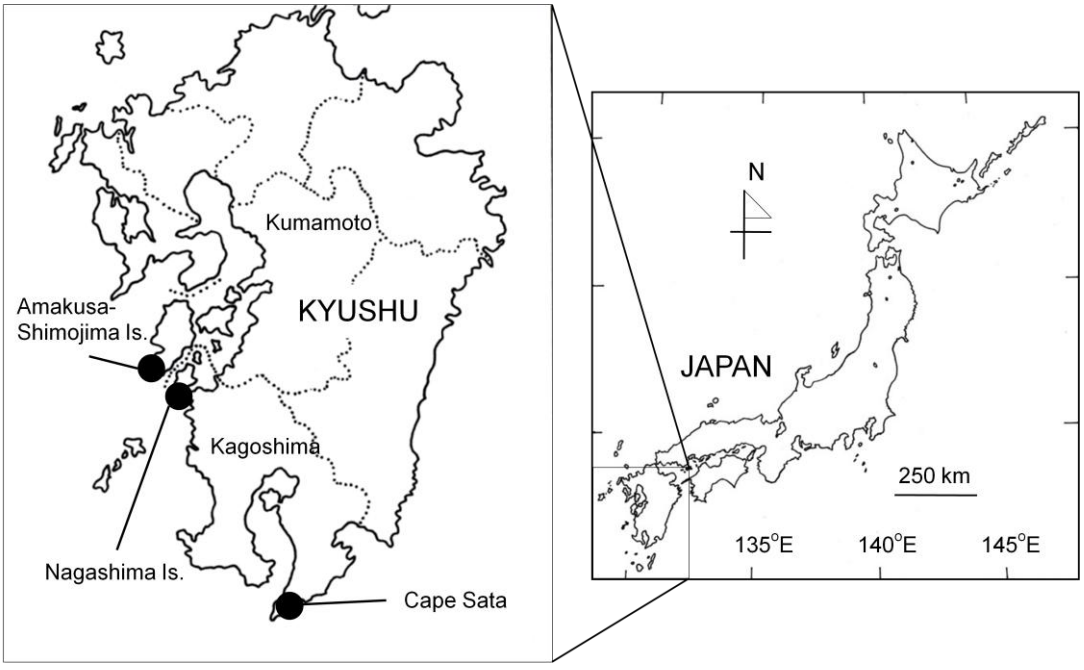

Fig. 3.2. Map of Kyushu Is., Japan showing the study sites of *Meristotheca coacta* and *M. papulosa*.

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 m^3) 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), I took measurements from 12:30 and 13:00 on July 2, 2011 just below the seawater surface (0 m), and at depths of 3, 5, 10, 20, 30, 40 and 50 m with light intensity data logger MDS-Mk-V/L (S/N200457, JFE-Advantech, Japan). Underwater temperature was also measured with light intensity data logger 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 July 7, 2010 just below the seawater surface (0 m), and at 5, 10, 15, 20 and 25 m depths by a light meter (LI-250 with spherical quantum sensor LI-193SA, Li-Cor, USA).

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

$$I_{\mathrm{D}} = I_0 \cdot \exp(-K \cdot D) \quad (1)$$

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

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 (Fig. 3.3.), Heinz Walz GmbH, Germany) using an incremental sequence of actinic illumination periods, with light intensities increasing in 21 steps from 0 to 1,078 μmol photons m^{-2} 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 ($\Phi_{PSII} = (F - F_m')/F_m'$, 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).

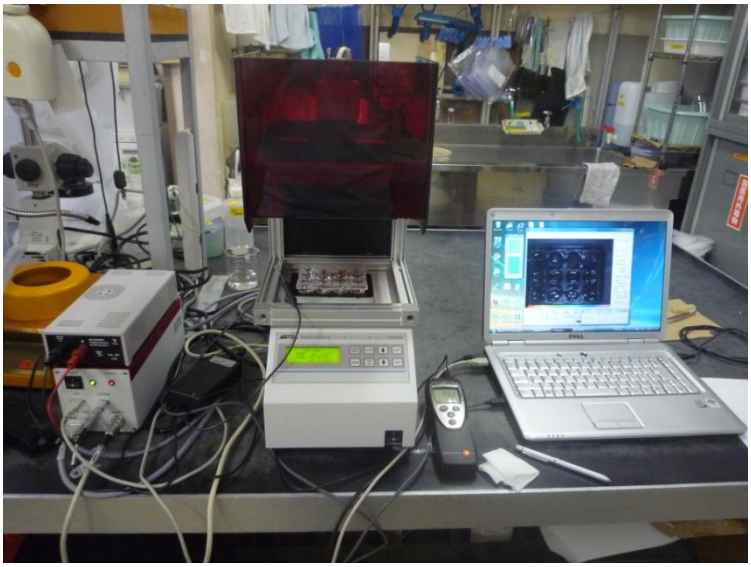

Fig. 3.3. Imaging-PAM used in this study.

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:

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

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

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

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 1000 samples for each chain (i.e., 4000 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 $rETR_{max}$,) link-function as appropriate. Two models were used to examine these relationships, a linear model, where θ ~ species + temperature + species × temperature and a quadratic model, where θ ~ species + temperature + species × temperature + temperature^2 + species × temperature^2.

RESULTS

Underwater temperature and PAR at the study sites

M. 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 2143 to 11 μmol photons m^{-2} s^{-1} on July 2, 2011. Near Nagashima Is., PAR at depths of 0 m to 25 m ranged from 248 to 10 μmol photons m^{-2} s^{-1} on July 7, 2010 (Fig. 3.4), during the measurements, we experienced fine clear skies at Cape Sata;

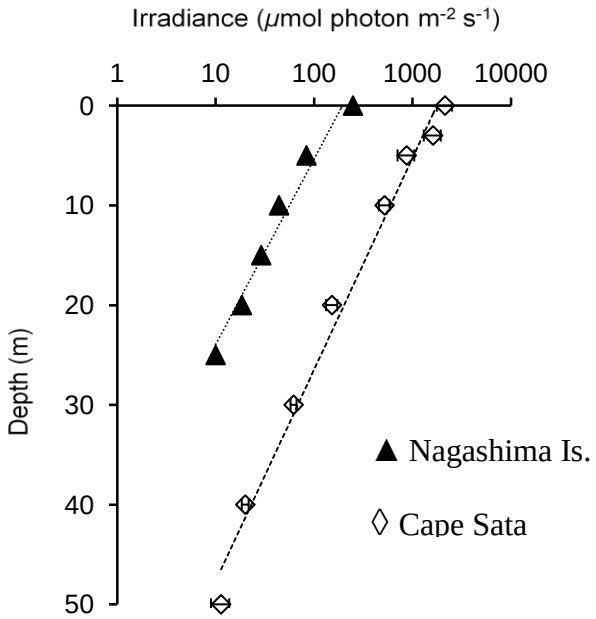

Fig. 3.4. Underwater photosynthetically active radiation, PAR (μmol photons m^{-2} s^{-1}) in Cape Sata (12:30 to 13:00, July 2, 2011) and Nagashima Is. (11:30 to 12:00, July 7, 2010).

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:

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

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

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

Table 3.1. Underwater temperature at Cape Sata measured on July 2, 2011 and estimated-underwater PAR* at Cape Sata and Nagashima Is. if the surface irradiance was 2000 or 2200 μ mol photon m^{-2} s^{-1}

Temperature (˚C)		Estimated Underwater PAR (μmol photon m-2 s-1)			
	Cape Sata	Cape Sata**		Nagashima***	
Depth (m)		2000	2200	2000	2200
3	24.9	1451	1596	1395	1535
5	24.1	1171	1288	1098	1207
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 July 2, 2011 (Sata) and July 7, 2010 (Nagashima).

*Cape Sata: $I_{(D)} = I_{(0)}\ e^{-0.107*D}$

**Nagashima Island: $I_{(D)} = I_{(0)}\ e^{-0.12*D}$

$I_{(D)}$: PAR at the objective depth (m)

$I_{(0)}$: PAR at the surface

D: objective depth (m)

Cape Sata and Nagashima Is. models were 1,717 μmol photons m^{-2} s^{-1} and 185 μmol photons m^{-2} s^{-1}, respectively.

In general, maximum irradiance at the coastal area at noon was around 2,000 to 2,200 μmol photons m^{-2} 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) μmol photons m^{-2} 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) μmol photons m^{-2} s^{-1}. For those of Nagashima Is., PAR ranged from 1,395 (1,535) to 55 (66) μmol photons m^{-2} 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˚Con July 2, 2011 (Table 3.1).

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. 3.5). At any given temperature and PAR, the rETR of *M. coacta* tended to be higher than that of *M. papulosa*. 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

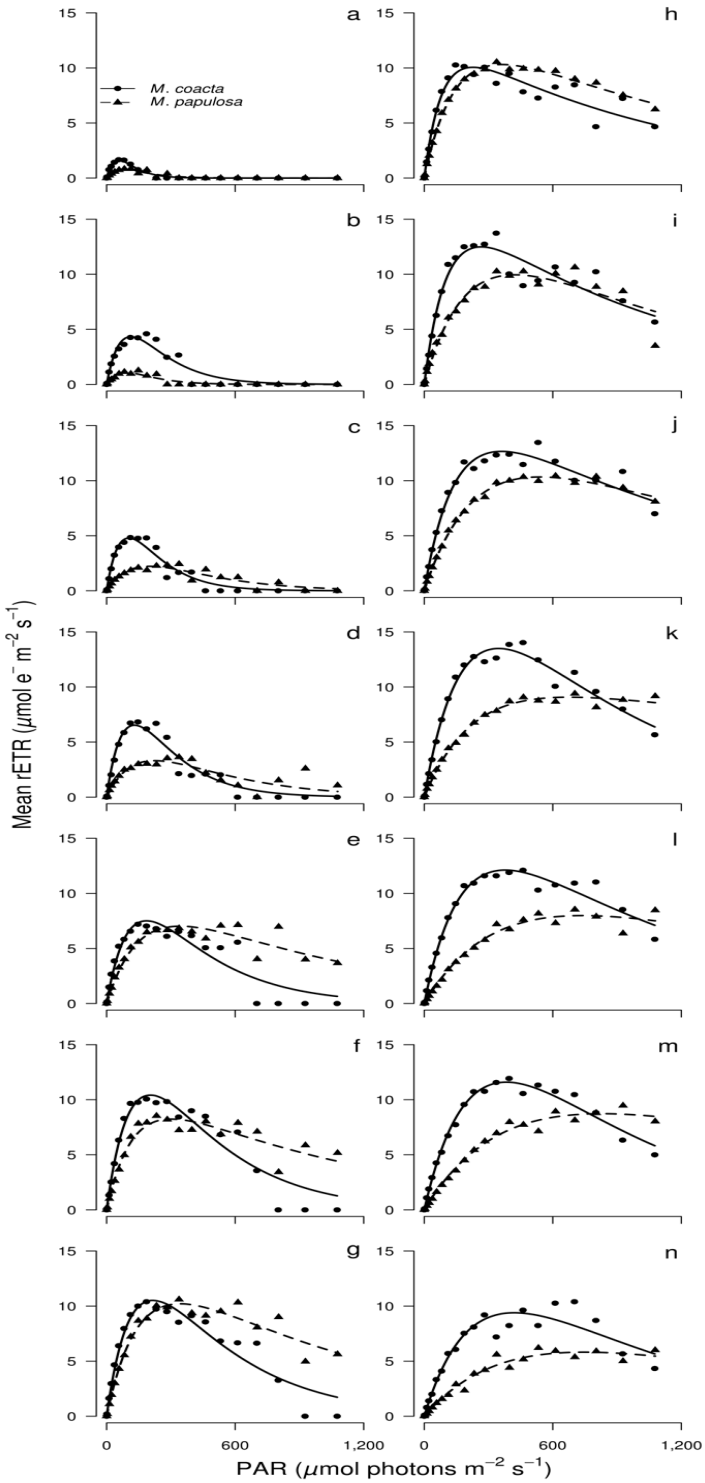

Fig. 3.5. The rapid light curves as determined by the hierarchical Bayesian analysis of *Meristotheca coacta* and *M. papulosa* determined over a temperature gradient of (a) 8 °C, (b) 10 °C, (c) 12 °C, (d) 14 °C, (e) 16 °C, (f) 18 °C, (g) 20 °C, (h) 22 °C, (i) 24 °C, (j) 26 °C, (k) 28 °C, (l) 30 °C, (m) 32 °C, (n) 34 °C. The solid and dash lines indicate the fitted model for *M. coacta* and *M. papulosa*, respectively.

PAR_{sat}, PAR_{opt}, and $rETR_{max}$. The parameters of the model as well as the derived estimates were then examined in detail using GLM (Fig. 3.5) 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 μmol e^- m^{-2} s^{-1} for *M. coacta* and 12.0 to 19.8 μmol e^- m^{-2} s^{-1} for *M. papulosa* over the range of temperatures examined and did not appear to be related to temperature (Fig. 3.6.a). The log-link gamma GLM fit to this data, revealed the insignificance of temperature versus species interactions ($F_{(1,24)} = 0.073$, $P = 0.7891$) and of temperature dependence ($F_{(1,26)} = 1.643$, $P = 0.2117$). However, a species effect was detected ($F_{(1,26)} = 55.102$, $P < 0.0001$), indicating that the values for *M. coacta* were significantly higher than that of *M. papulosa*. There was an estimated 15.1 μmol e^- m^{-2} 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 μmol e^- (μmol photons)$^{-1}$ and 0.065 to 0.156 μmol e^- (μmol photons)$^{-1}$, respectively and were dome-shaped (Fig. 3.6.b). Unlike the GLM for the parameter γ, a quadratic equation was fitted to α. Species effects were significant in the

model ($F_{(1,26)}$ = 209.338, P < 0.0001)), where α was greater for *M. coacta*. The value of α also significantly varied with the square of the temperature ($F_{(1,24)}$ = 144.856, 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 μmol e^- (μmol photons)$^{-1}$ and 0.078 μmol e^- (μmol photons)$^{-1}$ for *M. coacta* and *M. papulosa*, respectively.

The mean values of the photoinhibition coefficient (β) of these species ranged from 0.014 to 0.381 μmol e^- (μmol photons)$^{-1}$ for *M. coacta* and from 0.004 to 0.157 μmol e^- (μmol photons)$^{-1}$ for *M. papulosa* and were U-shaped in nature (Fig. 3.6.c). This parameter was also analyzed using the quadratic GLM, where the quadratic term was significant ($F_{(1,24)}$ = 19.773, P = 0.0002). No interactions were evident ($F_{(1,23)}$ = 0.939, P = 0.3430), but species was an important factor in the model ($F_{(1,26)}$ = 37.822, 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 μmol e^- (μmol photons)$^{-1}$ for *M. coacta* and 0.009 μmol e^- (μmol photons)$^{-1}$ for *M. papulosa*.

The mean values of $rETR_{max}$ could also be examined using a quadratic model (Fig. 3.6.d), and these values increased from low temperatures and peaked near 25.5 °C.

Indeed, the quadratic terms were significant ($F_{(1,24)}$ = 188.346, P < 0.0001) as was the species effect ($F_{(1,26)}$ = 25.249, P < 0.0001). Interactions among species and temperature was also significant ($F_{(1,23)}$ = 8.920, P = 0.0068). A more detailed examination of the model indicated that $rETR_{max}$ peaked with a value of 13.3 μmol e^{-} m^{-2} s^{-1} at 26.0 °C for *M. coacta* and was 11.7 μmol e^{-} m^{-2} 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. 3.6.e). 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. 3.6.f), 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. papuloa* was generally higher and more sensitive to temperature, compared to *M. coacta*.

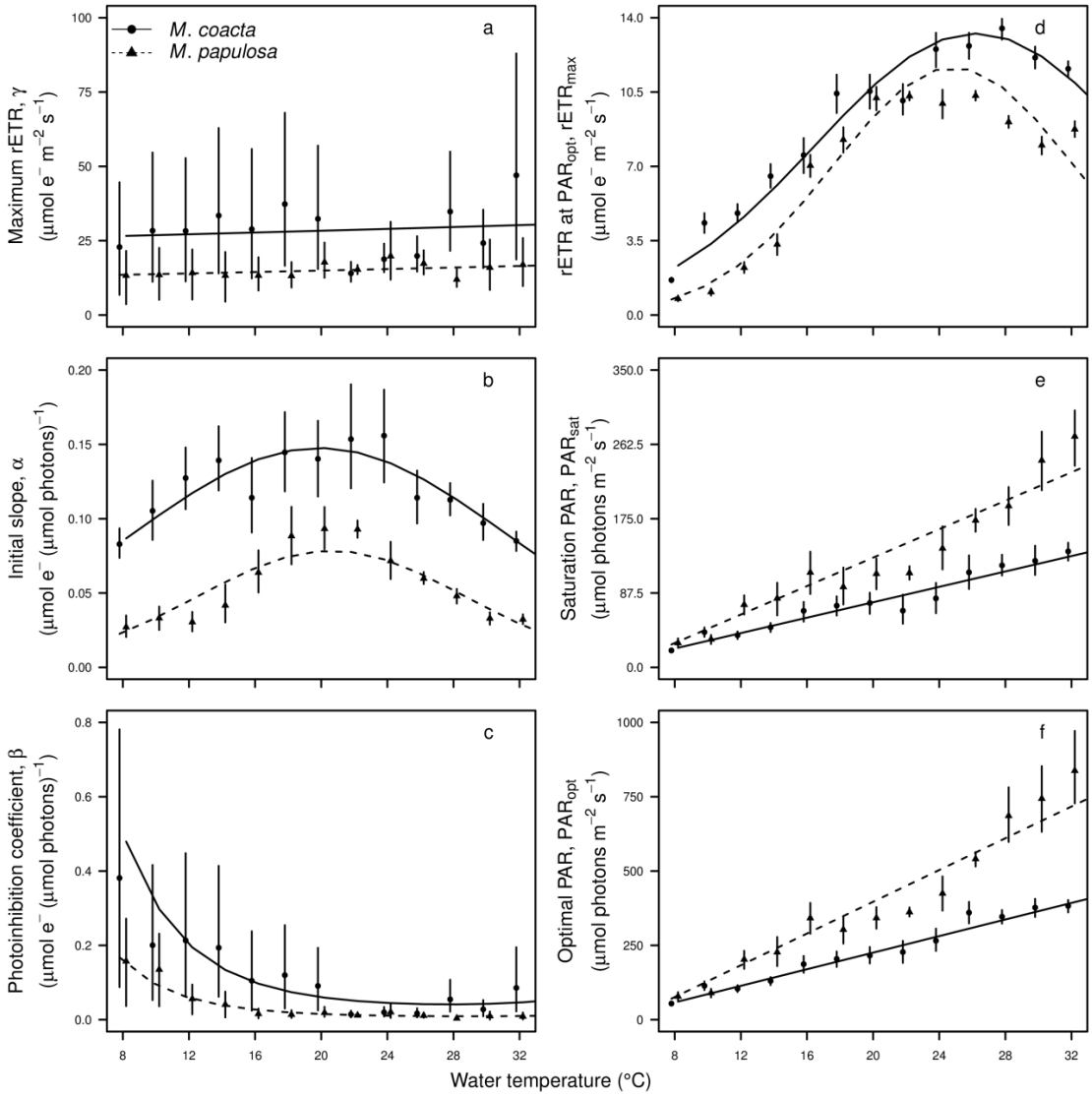

Fig. 3.6.The model parameters determined by the hierarchical Bayesian model express a variety of temperature dependence for both *Meristotheca coacta* and*M. papulosa*. (a) The maximum relative electron transport rates (rETR) in absence of photoinhibition, γ, are independent of temperature. (b) The initial 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), $rETR_{max}$, 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.

Estimated equation coefficient value and the equations of the correlation between the photosynthetic parameters and temperatures (t) were summarized in Table 3.2 and 3.3. And the estimated values of PAR_{sat} and PAR_{opt} at some temperatures were presented in Table 3.4.

Table 3.2. Estimated equation coefficient of correlation between photosynthetic parameters and temperatures models of *Meristotheca coacta* and *Meristotheca papulosa* done by using generalized linear model (GLM).

	Estimate	Std. Error	t value	Pr(>|t|)
The parameter values for the $rETR_{max}$ In the absence of Photoinhibition (γ) – temperature model				
(Intercept)	25.4691	4.7800	5.3282	0.0000
Speciespapulosa	-13.0811	5.3812	-2.4309	0.0229
Temperature	0.1469	0.2186	0.6718	0.5081
Speciespapulosa : temperature	-0.0186	0.2471	-0.0755	0.9405
The parameter values for the α – temperature model				
(Intercept)	-0.0237	0.0189	1.2529	0.2228
Speciespapulosa	-0.0817	0.0153	-5.3318	0.0000
Temperature	0.0116	0.0015	7.7573	0.0000
I (temperature^2)	-0.0003	0.0000	-9.4485	0.0000
Speciespapulosa : temperature	0.0009	0.0006	1.4967	0.1481
The parameter values for the β – temperature model				
(Intercept)	0.3203	0.0590	5.4337	0.0000
Speciespapulosa	-0.1040	0.0472	-2.2052	0.0377
Temperature	-0.0172	0.0038	-4.5168	0.0002
I (temperature^2)	0.0003	0.0001	3.7223	0.0011
Speciespapulosa : temperature	0.0023	0.0017	1.3548	0.1886
The parameter values for the $rETR_{max}$ (at PAR_{opt}) – temperature model				
(Intercept)	-7.5856	1.1404	-6.6514	0.0000
Speciespapulosa	-0.2476	0.6924	-0.3575	0.7240
Temperature	1.3557	0.1504	9.0132	0.0000
I (temperature^2)	-0.0234	0.0039	-5.9292	0.0000
Speciespapulosa : temperature	-0.1079	0.0569	-1.8945	0.0708
The parameter values for the PAR_{sat} – temperature model				
(Intercept)	-13.2050	4.8644	-2.7146	0.0121
Speciespapulosa	-26.8622	8.2975	-3.2374	0.0035
Temperature	4.5362	0.3442	13.1776	0.0000
Speciespapulosa : temperature	3.8415	0.6375	6.0257	0.0000
The parameter values for the PAR_{opt} – temperature model				
(Intercept)	-50.5944	12.5097	-4.0444	0.0005
Speciespapulosa	-92.3212	21.6407	-4.2661	0.0003
Temperature	13.9424	0.9203	15.1498	0.0000
Speciespapulosa : temperature	12.7683	1.7484	7.3030	0.0000

Note: The (intercept), temperature and/or temperature^2 value showed in this table were basically written for *M. coacta* and *M. papulosa*. If species papulosa and Speciespapulosa:temperature value is statistically significant different, intercept of *M. papulosa* was intercept + Speciespapulosa and temperature value of *M. papulosa* was Temperature + Speciespapulosa:temperature.

Table 3.3. The equations of relation between photosynthetic parameters (γ, α, β, $rETR_{max}$, PAR_{sat}, PAR_{opt}) and temperature (t) ranged from 8 to 34 °C done by using generalized linier model (GLM)

Parameter	*M. coacta*	*M. papulosa*
γ	$\gamma = 25.4691 + 0.1469t$	$\gamma = 12.388 + 0.1283t$
Initial slpoe (α)	$\alpha = 0.0237 + 0.0116t - 0.0003t^2$	$\alpha = -0.058 + 0.0125t - 0.0003t$
Photoinhibition (β)	$\beta = 0.3203 - 0.0172t + 0.0003t^2$	$\beta = 0.2163 - 0.0149t + 0.0003t^2$
Maximum rETR (rETRmax)	$rETR_{max} = -7.5856 + 1.3557t - 0.0234t^2$	$rETR_{max} = -7.8332 + 1.2478t - 0.0234t^2$
Saturating PAR (PARsat)	$PAR_{sat} = -13.2050 + 4.5362t$	$PAR_{sat} = -40.0674 + 8.3777t$
Optimum PAR (PARopt)	$PAR_{opt} = -50.5944 + 13.9424t$	$PAR_{opt} = -142.9156 + 26.7107t$

Table 3.4. Estimated Saturating PAR and Maximum PAR of *M. coacta* and *M. papulosa* at some temperatures calculated by using equations derived from generalized linier model

Temperature (°C)	*M. coacta*		*M. papulosa*	
	PARsat	*PARopt*	*PARsat*	*PARopt*
8	23.08	60.94	26.95	70.77
10	32.16	88.83	43.71	124.19
12	41.23	116.71	60.47	177.61
14	50.30	144.60	77.22	231.03
16	59.37	172.48	93.98	284.46
18	68.45	200.37	110.73	337.88
20	77.52	228.25	127.49	391.30
22	86.59	256.14	144.24	444.72
24	95.66	284.02	161.00	498.14
26	104.74	311.91	177.75	551.56
28	113.81	339.79	194.51	604.98
30	122.88	367.68	211.26	658.41
32	131.95	395.56	228.02	711.83
34	141.03	423.45	244.77	765.25

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. 3.6.b). Meanwhile, the photoinhibition coefficient (β) of the two species decreased from low temperatures (Fig. 3.6.c), and $rETR_{max}$ increased from low temperatures to a peak between 25 to 26 °C (Fig. 3.6.d). These results suggest that the optimal temperatures of these two species is most likely with 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 parameter 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 μmol e^{-} m^{-2} s^{-1} for *M. coacta* and 11.1 μmol e^{-} m^{-2} 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 μmol photons m^{-2} s^{-1}. The initial slope (α) of *M. coacta* was always higher than that of *M. papulosa* at each temperature condition examined (Fig. 3.6.b). However, the PAR_{sat} and PAR_{opt} for the former were always lower than those of the latter (Fig. 3.5.e and 3.5.f), suggesting that *M. coacta* can photosynthesize and survive under lower levels of PAR. We guess that this difference is related to their habits, because the prostrated appearance of *M. coacta*, found growing on the rocks are sometimes shaded by *M. papulosa* and other organisms (Fig 3.2).

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 the 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 (Guiry, 2011), and the prevalence of *M. coacta* in Japan (Yoshida, 1998), Korea (Lee & 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. 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* (Fig. 3.6.e, 3.6.f) measured in this study increased with increasing of water temperature (Collins and Boyen, 1982; Palmisano *et al*., 1987; Henley, 1992, 1993). I 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 μmol photons m^{-2} 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).

M. 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. 3.4, Table 3.1) corresponded to the mean values for the PAR_{sat} estimated at the temperatures from 18 to 22 ˚C (Fig. 3.6.e). These temperatures also corresponded to the temperatures measured at the depth of the study site (Table 3.1). I 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 temperatures 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 summer of 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 the results I suggest that either of the species can be successfully cultivated from April to August in this region.

CHAPTER 4: EFFECT OF TEMPERATURE AND LIGHT ON THE PHOTOSYNTHESIS OF CULTURED *Eucheuma denticulatum* AND *Kappaphycus* sp. (Sumba strain) FROM INDONESIA

INTRODUCTION

The red algal genera *Eucheuma* and *Kappaphycus* (Solieriaceae) are considered commercially important commodities due to their production of *carrageenan*, which is used in the pharmaceutical, cosmetics, textile, and food industries as a firming agent, emulsifier, coagulating agent, and filling material (Waaland *et al.*, 1981). They are largely cultivated in tropical areas especially in South East Asia, particularly in Indonesia and the Philippines (Bixler and Porse, 2010). It is relevant to note that Indonesia has become a major exporter of *Eucheuma* raw materials and supplies a large foreign demand for iota-carrageenan (Adnan and Porse, 1987), indeed the mariculture of genera such as *Kappaphycus* and *Eucheuma* supplies most of the world's *carrageenan* and carrageenan-like products (Dawes *et al*., 1994). For example, *Betaphycus gelatinus* (as *Eucheuma gelatinae*) has been commercially cultivated in China since 1960 (Waaland *et al.*, 1981), and the commercial cultivation of *Kappaphycus alvarezii* 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 and Braud, 1993), and other regions of Vietnam (Ohno *et al*.,

1996) .

These successes were apparently due to similarities in environmental conditions (*e.g*., water temperature and irradiance) between the transplant sites and the host sites where *Eucheuma* was originally collected (Ohno *et al*., 1994). Indeed, temperature and irradiance are considered the fundamental environmental factors influencing photosynthetic processes (*i.e*., the conversion of light to chemical energy, such as sugars and other organic molecules) of *Eucheuma* and *Kappaphycus*. Therefore, detailed information regarding how photosynthetic processes respond to water temperature and irradiance would serve to enhance and optimize present cultivation systems.

Some strains under the name of *Kappaphycus alvarezii* or related species are cultured in Indonesia such as the *Sakol, Tambalang, cottonii* and *Sumba*. The *Sumba* strain (*Kappaphycus* sp.) is believed to originate from Sumba Island in Indonesia, while the *Sakol* and *Tambalang* strains were brought over from the Philippines in the 1990s.

Since the 1970's, a number of studies on *Eucheuma* and related genera, from a variety of locations throughout the world (*e.g*., Dawes *et al*., 1974; Paula *et al*. 2001, 2002; Hung *et al*., 2009), have greatly contributed towards elucidating their characteristics of growth and *carrageenan* content, and have led also contributed to

conservation of natural resources and the enhancement of cultivation techniques and supply. In the genera *Eucheuma* and *Kappaphycus*, *E. denticulatum* and *K. alvarezii* were the most commonly used species in experiments (Dawes *et al*., 1994; Ohno *et al*.1994; Wobeser *et al*, 2001), and studies on photosynthesis was mainly conducted by measuring dissolved oxygen evolution under relatively low conditions of irradiance (e.g., < 650 μmol photons m^{-2} s^{-1}; Wobeser *et al*, 2001). In Indonesia, the *Sumba* Strain, which is a strain of *Kappaphycus*, and *E. denticulatum* are the most prominent species cultivated, however little is known regarding their physiology. Therefore, any information regarding the physiology of this strain could improve the current cultivation system for Indonesian *Eucheuma/Kappaphycus* aquaculture. Consider that the optimum temperature and light requirements for photosynthesis have yet to be determined for these strains.

Since the late 1990's, pulse amplitude modulated (PAM)-chlorophyll fluorometry has been used for some seagrasses as well as many terrestrial plants (Beer *et al.*, 1998; Ralph *et al*., 1998, 2006; Beer and Björk, 2000; Kuster *et al*. 2007; Aldea *et al*., 2006) as a quick and efficient way of evaluating the photosynthetic response of whole intact plants. More recently, PAM has been used in aquatic research of seaweeds to elucidate photosynthetic response (Gevaert *et al*., 2002). Clearly, PAM would be an

efficient way of clarifying the response of cultured plants of *Eucheuma/Kappahycus*, and a battery of tests could be designed to clarify the optimal temperature and irradiance conditions that would maximize photosynthesis (*i.e*., growth) and also be used to diagnose the physiological condition of cultivated seaweeds (*i.e*., health).

In this study we focused on elucidating the temperature and irradiance conditions needed to promote photosynthesis in cultured *Eucheuma denticulatum* and *Kappaphycus* sp. (Sumba Strain) from Indonesia using PAM-Chlorophyll fluorometry. We use this technology to perform the first trial study of Indonesian *Eucheuma/Kappaphycus* photobiology, with the hope that this knowledge will lead to an advance in cultivation output and efficiency of these species.

MATERIALS AND METHODS

Specimen collection and stock maintenance

Cultured specimens of *Eucheuma denticulatum* and *Kappaphycus* sp. (Sumba starin) (Fig. 4.1) were collected at farming area in Funaga, South Sulawesi, Indonesia (5˚34’56”N, 119˚27’42”E) on August 15, 2010. Cultured specimens that were attached to a rope, were collected by authors and stored in 1,000 ml plastic bag in a cool box at temperature of approximately 24 °C and they transported to the laboratory in the T/S

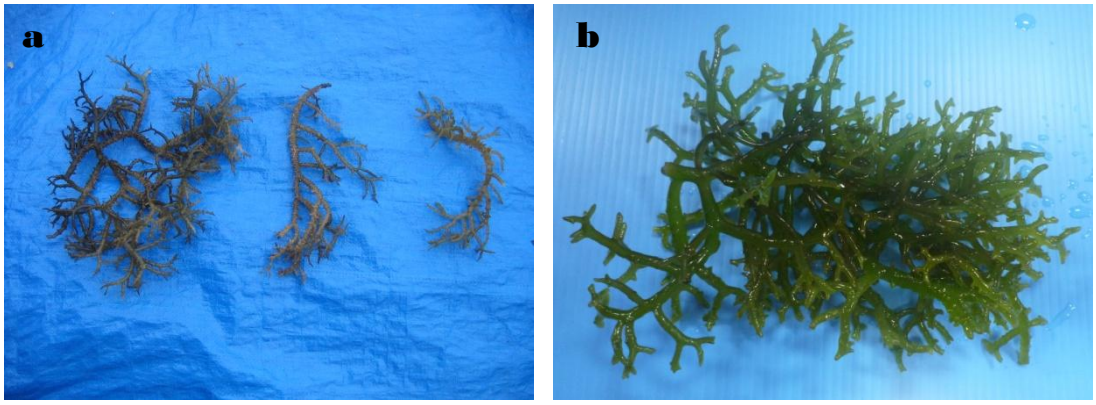

Fig.4.1 Specimens of cultured *Eucheuma denticulatum* (a) and cultured *Kappaphycus* sp. (Sumba strain) (b) in Funaga, South Sulawesi, Indonesia.

"*Kagoshima Maru*" a research vessel of Faculty of Fisheries, Kagoshima University, Japan, that was anchored in the *Pelabuhan Benoa*, Bali Indonesia. The specimens were maintained in a tank (1.0 × 1.0 × 0.5 m^3) containing seawater of salinity at 33 PSU, pH of 8.0, irradiance of 90μmol photons m^{-2} s^{-1} and temperature 24 °C under 12:12 light: dark cycle during the experiments. Voucher herbarium specimens of the two species were deposited in the herbarium of Marine Botany, Kagoshima University Museum (*KAG*) for future species identification (especially, *Kappaphycus* sp.).

Underwater temperature and PAR at specimens collecting site

We measured photosynthetic active radiation (PAR) and sea surface temperatures in the sample collection area. Light meter model LI-250 (LI-COR, USA)

and thermometer (YSI model 85, USA) were used for measuring PAR and temperature, respectively. PAR and temperature levels were measured at cultivation area of 300 m from shore line with depth of 4 m, 700 m from shore line with depth of 5 m and 1000 m from shore line with depth of 5 m. 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

Rapid Light Curves (RLCs)

Rapid light curves (RLCs) were generated by running the standard algorithm of the pulse amplitude modulated (PAM)-chlorophyll fluorometer (Diving-PAM (Fig. 4.2), Heinz Walz GmbH, Germany) using an incremental sequence of actinic illumination periods, with light intensities increasing in 9 steps from 0 to 1,000 μmol photons $m^{-2}s^{-1}$ 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 ($\Phi_{PSII} = (F - F_m')/F_m'$, 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).

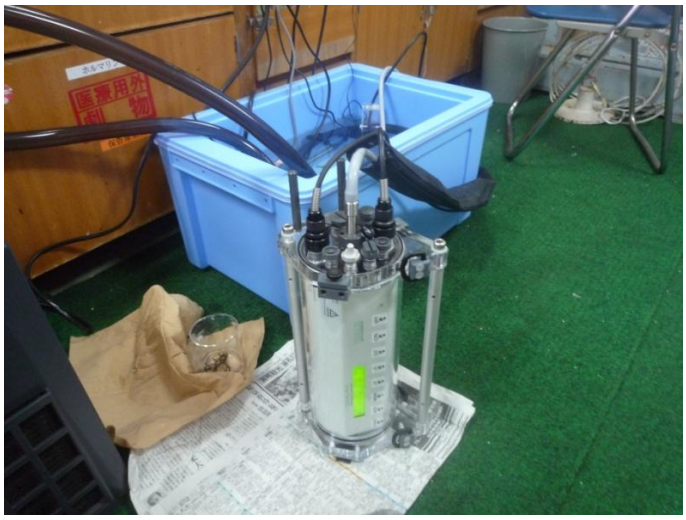

Fig. 4.2 Diving-PAM used in this study.

Temperature and light effect on photosynthetic parameters

From each specimen, 2 cm long portions of the thalli were placed in a 50 x 40 x 50 cm of plastic tank with seawater, allowing for 4 replicates for each species. Temperature was adjusted and maintained by temperature controlled water bath (Coolnit CL-80F, Taitec, Inc., Tokyo). Water temperature in the tank was 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 9 PAR levels, for every 2 °C increment temperature ranging from 16 to 34°C.

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):

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

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 maximal.

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

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 can be estimated from the first real root:

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

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

$$PAR_{\text{sat}} = \frac{rETR_{\max}}{\alpha} \quad (5)$$

Statistical analysis

Statistical analyses of photosynthetic parameters of γ, α and β were done using *R* (R Development Core Team, 2011) and OpenBUGS (Thomas *et al*., 2006). The parameters were examined by fitting the RLCs to non linier model (Eq. 2) using hierarchical Bayesian methods.

Model formulation and selection of the relationship between the estimated parameters and experimental water temperature were also examined based on Bhujel (2009). 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. General linier model was used to examine temperature and species effect and also 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 linier model were analyzed by using SPSS v.17 (SPSS Inc.).

RESULTS

Underwater temperature and PAR at specimens collecting site

PAR levels which were measured at collection site of cultured *Eucheuma*

denticulatum and *Kappaphycus* sp. with depth of 0.5 m to 5 m ranged from 1,448 to 393 μmol photon m^{-2} s^{-1} (300 m from shore line), 1,482 to 109 μmol photon m^{-2} s^{-1} (700 m from shore line) and 1,213 to 138 μmol photon m^{-2} s^{-1} (1,000 m from shore line), while average sea surface temperature level were recorded at 29 °C (Fig. 4.3). The

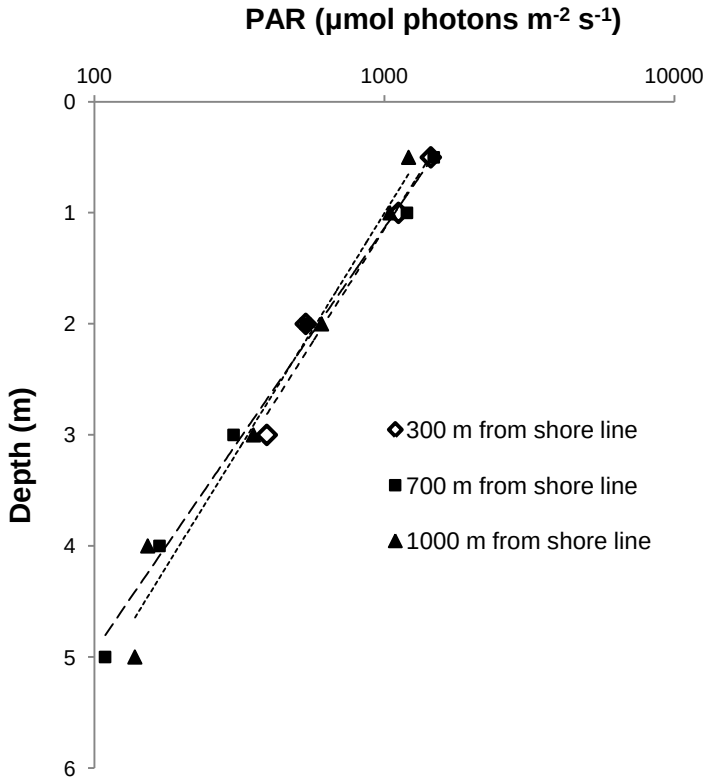

Fig. 4.3. Underwater photosynthetic active radiation, PAR (μmol photons m^{-2} s^{-1}) in Funaga, South Sulawesi, Indonesia (11:00 to 12:00, sunny day, August 4, 2011).

Beer-Lambert equation was fitted to PAR measurements taken at the study sites using a linear regression on the log-transformed PAR and was determined to be:

300 m from shore line: $I_{(D)} = 1{,}848.4\ e^{-0.543 \cdot D}$ ($R^2 = 0.9713$)

400 m from shore line: $I_{(D)} = 1{,}964.8 e^{-0.6 \cdot D}$ ($R^2 = 0.9925$)

700 m from shore line: $I_{(D)} = 1{,}666.3\ e^{-0.53 \cdot D}$ ($R^2 = 0.9752$)

where, the extinction coefficients (K) determined for waters at 300, 400 and 700m from shore line were 0.543, 0.6 and 0.53, respectively and the coefficient of surface PAR for their models were 1,848.4 μmol photons m^{-2} s^{-1}; 1,964.8 μmol photons m^{-2} s^{-1} and 185 μmol photons m^{-2} s^{-1}, respectively. Estimated underwater PAR was showed in Table 4.1.

Table 4.1. Estimated-underwater PAR (µmol photons m^{-2} s^{-1}) at Funaga, South Sulawesi, Indonesia measured on August 4, 2011, if the surface irradiance was 2000 µmol photons m^{-2} s^{-1}

Depth (m)	Distance from shore line		
	300 m	400 m	700 m
0	2000	2000	2000
0.5	1524.5	1481.6	1534.4
1	1162.0	1097.6	1177.2
2	675.1	602.4	692.9
3	392.3	330.6	407.9
4		181.4	240.1
5		99.6	141.3

Rapid Light Curves (RLCs)

Generally, the rapid light curves of these species showed an increasing until reaching some asymptote as common occurred in photosynthesis – irradiance curves

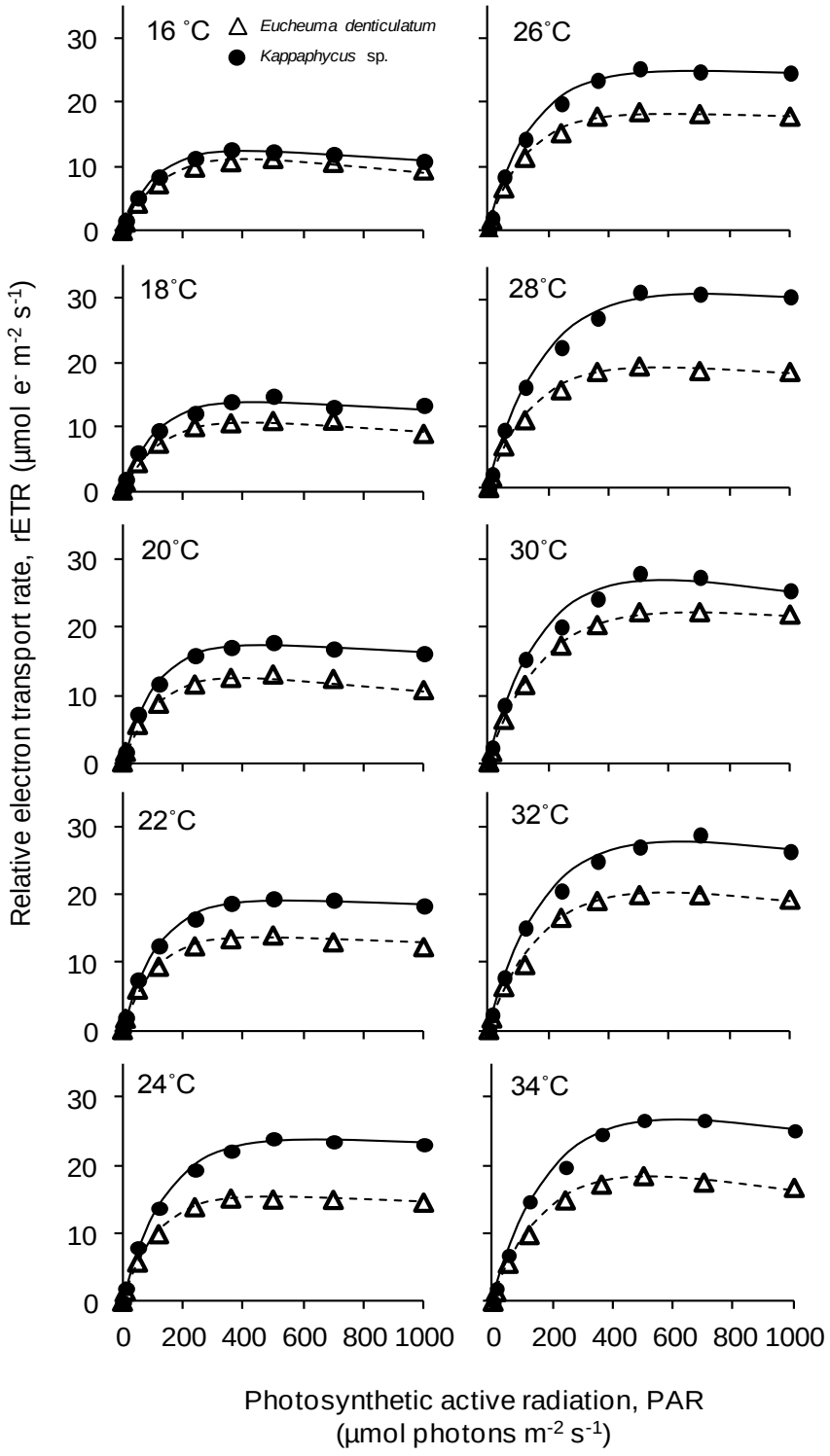

Fig. 4.4. The rapid light curves of rETR as determined by the hierarchical Bayesian analysis of *Eucheuma denticulatum* and *Kappaphycus* sp. determined over a temperature gradient of 16 °C, 18 °C, 20 °C, 22 °C, 24 °C, 26 °C, 28 °C, 30 °C, 32 °C, 34 °C. The dash and solid lines indicate the fitted model for *E. denticulatum* and *Kappaphycus* sp., respectively, n= 4.

(*P-I* curve) and the photo-inhibition were not clear until PAR of 1,000 μmol photons m^{-2} s^{-1} (Fig. 4.4).

At any given temperature and PAR, the rETR of *Kappaphycus* sp. tended to be higher than that of *E. denticulatum*. By fitting Eq. 3 to the results using hierarchical Bayesian methods, we were able to elucidate the parameters of the model (γ, α, β) across all water temperatures, as well as derive estimates of $rETR_{max}$, PAR_{sat} and PAR_{opt}.

Effect of temperature on the photosynthetic parameters

The mean values of the maximum rETR in the absence of photoinhibition (γ), ranged from 13.1 to 25.0 μmol e^- m^{-2} s^{-1} for *E. denticulatum* and 14.3 to 34.6 μmol e^- m^{-2} s^{-1} for *Kappaphycus* sp. over the range of temperatures examined and they monotonically increased with increasing temperature (Fig. 4.5.a). The curve models of γ for *E. denticulatum* and *Kappaphycus* sp. were linier against temperatures ($F_{(1,39)}$ = 112.992, P <0.001 and ($F_{(1,39)}$ = 207.365, P <0.001, respectively). The relation model of γ against temperatures (t) and could be described by equation: $\gamma = 0.127 + 0.753$ t (R^2 = 0.748) for *E. denticulatum* and $\gamma = -5.243 + 0.73$ t (R^2 = 0.845) for *Kappaphycus* sp. Temperatures and species had a significant effect on γ ($F_{(9,80)}$ = 54.905, P < 0.001 and $F_{(9,80)}$ = 148.159, P < 0.001, respectively), there was also an interaction between

temperature and species ($F_{(1,80)}$ = 4.914, P < 0.001), which indicate that γ for *Kappaphycus* sp. significantly higher and more sensitive, compare to *E. denticulatum.*

The mean values of the initial slope (α) of *E. denticulatum* and *Kappaphycus* sp. ranged from 0.076 to 0.148 μmol e^- (μmol photons)$^{-1}$ and 0.123 to 0.181 μmol e^- (μmol photons)$^{-1}$, respectively and were dome-shaped (Fig. 4.5.b). The relation model between temperature (t) and α of *E. denticulatum* and *Kappaphycus* sp. were quadratic ($F_{(2,39)}$ = 51.151, P <0.001 and ($F_{(2,39)}$ = 31.098, P <0.001, respectively) and they could be described by equation: α = - 0.2726 + 0.0313 t - 0.0006 t^2 (R^2 = 0.734) for *E. denticulatum* and α = - 0.1651 + 0.0256 t - 0.0005 t^2 (R^2 = 0.627) for *Kappaphycus* sp. Based on these models, highest α of *E. denticulatum* and *Kappaphycus* sp. were 0.135 μmol e^- (μmol photons)$^{-1}$ and 0.163 μmol e^- (μmol photons)$^{-1}$occurred at temperature 26.1 °C and 25.6 °C, respectively. Hence, for α, 95% of the maximum would be 0.128 μmol e^- m^{-2} s^{-1} for *E. denticulatum* and 0.154 μmol e^- m^{-2} s^{-1} for *Kappaphycus* sp., which leads to temperature range of 22.6 – 29.6 °C and 21.5 – 29.7 °C, respectively. The α values were significantly affected by temperatures ($F_{(9,80)}$ = 16.860, P < 0.001), while the interactions between temperature and species were insignificant ($F_{(1,80)}$ = 4.914, P = 0.976). However, a species effect was detected ($F_{(9,80)}$ = 149.863, P < 0.001), indicating that α values of *Kappaphycus* sp. were significantly higher than *E. denticulatum*. There

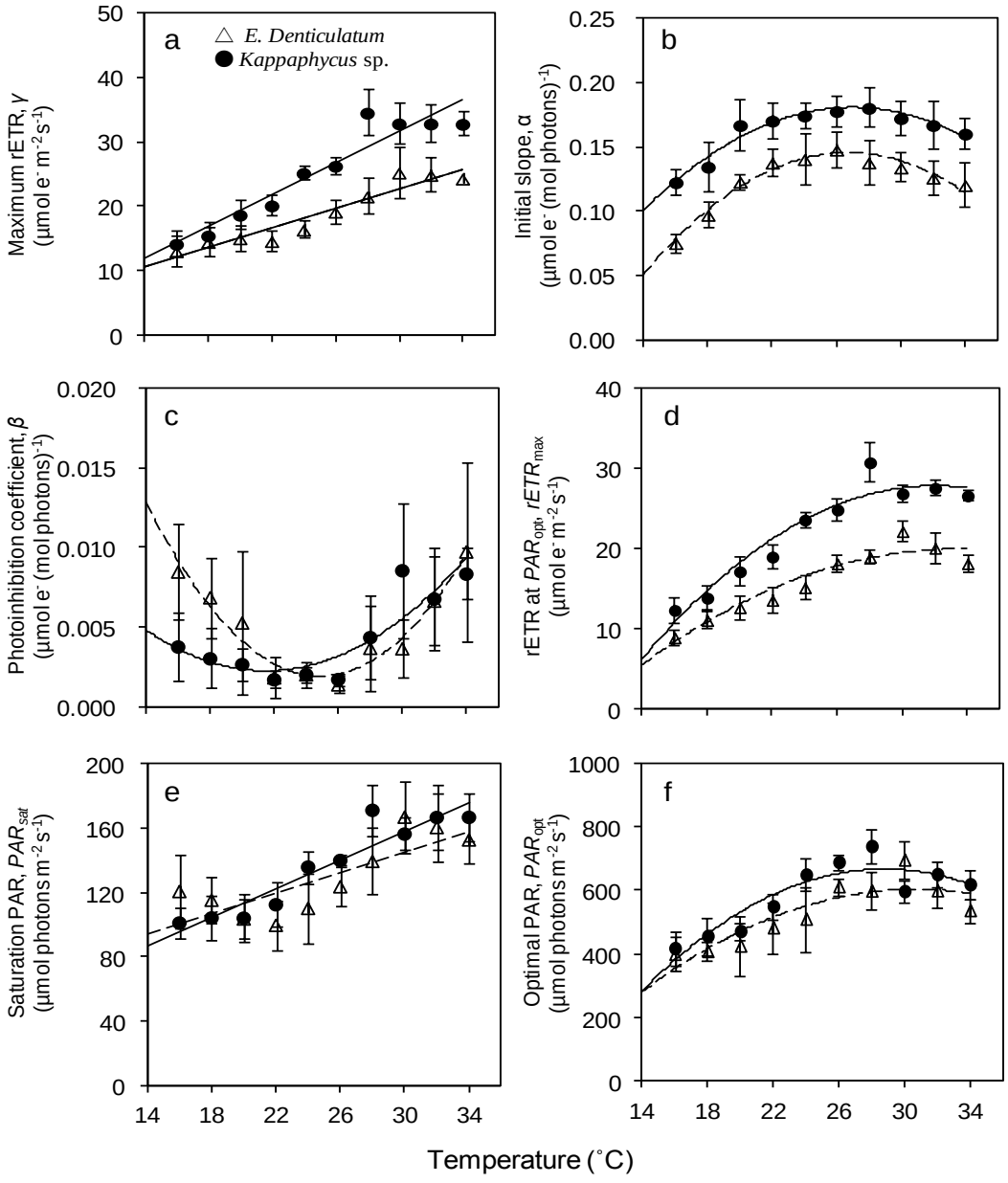

Fig. 4.5. The photosynthetic parameters at some temperature levels of cultured *Eucheuma denticulatum* (△) and *Kappaphycus* sp.(●) and the curve estimation showing the relation between photosynthetic parameters and temperatures of cultured *E. denticulatum* (gray lines) and *Kappaphycus* sp. (solid lines). (a) The maximum relative electron transport rates (rETR) in absence of photoinhibition, γ. (b) The initial slope of rapid light curves, α. (c) The photoinhibition coefficient, β. (d) The maximum rETR that was observed when photosynthetic active radiation (PAR) was optimum, $rETR_{max}$. (e) The PAR at which rETR rates begin to saturate, PAR_{sat}. (f) The PAR at which maximum rETR was observed, PAR_{opt}. Bars were the standard deviation, n = 4.

was an estimated 0.03 μmol e^- (μmol photons)$^{-1}$ difference in the parameter estimates of the maximum α rate between these species.

The mean values of the photoinhibition coefficient (β) of these species ranged from 0.001 to 0.010 μmol e^- (μmol photons)$^{-1}$ for *E. denticulatum* and from 0.002 to 0.09 μmol e^- (μmol photons)$^{-1}$ for *Kappaphycus* sp. and were U-shaped in nature (Fig. 4.5.c). This parameter had quadratic relationship model with temperatures (t) for both *E. denticulatum* ($F_{(2,39)}$ = 18.921, P < 0.001) and *Kappaphycus* sp. ($F_{(2,39)}$ = 19.164, P < 0.001) and they could be described by equation: β = 0.0589 - 0.0046 t + 0.0000918 t^2 (R^2 = 0.506) for *E. denticulatum* and β = 0.0232 - 0.0019 t + 0.0000452 t^2 (R^2 = 0.509) for *Kappaphycus* sp. A minima β could be estimated at temperature near 25.0 °C and 21.0 °C for *E. denticulatum* and *Kappaphycus* sp., respectively. In case of β, 95% of the minimum value would be 0.00139 μmol e^- m^{-2} s^{-1} for *E. denticulatum* and 0.00342 μmol e^- m^{-2} s^{-1} for *Kappaphycus* sp., which leads to temperature range of 24.1 – 26.0 °C and 19.2 – 22.8 °C, respectively. Although temperatures had significant effect on β ($F_{(9,80)}$ = 7.065, P < 0.001), these species was not a significant important factor ($F_{(1,80)}$ = 1.304, P = 0.258) , and the interactions were not clear ($F_{(9,80)}$ = 2.174, P = 0.037).

The mean values of $rETR_{max}$ of *E. denticulatum* and *Kappaphycus* sp. ranged from 9.02 to 22.26 μmol e^- m^{-2} s^{-1} and 12.44 to 30.84 μmol e^- m^{-2} s^{-1}, respectively (Fig.

4.5.d). A model which describe a relation between temperature and $rETR_{max}$ of *E. denticulatum* and *Kappaphycus* sp. were quadratic ($F_{(2,39)}$ = 100.046, P <0.001 and ($F_{(2,39)}$ = 141.720, P <0.001), respectively. The relation between temperature (t) and $rETR_{max}$ could be described by equation: $rETR_{max}$ = - 22.991 + 2.576 t - 0.039 t^2 (R^2 = 0.844) for *E. denticulatum* and *rETRmax* = - 40.590 + 4.275 t – 0.067 t^2 (R^2 = 0.885) for *Kappaphycus* sp. Based on these models, the maximum *rETRmax* of *E. denticulatum* and *Kappaphycus* sp. was reached 19.5 μmol e^- m^{-2} s^{-1} and 27.6 μmol e^- m^{-2} s^{-1} occurred at temperature 31.1 °C and 31.5 °C, respectively. Moreover, for α, 95% of the maximum would be 18.6 μmol e^- m^{-2} s^{-1} for *E. denticulatum* and 26.2 μmol e^- m^{-2} s^{-1} for *Kappaphycus* sp., which leads to temperature range of 28.0 – 34.0 °C and 27.4 – 34.0 °C, respectively. The *rETRmax* values were significantly affected by temperatures ($F_{(9,80)}$ = 111.081, P < 0.001), and there were interactions between temperature and species ($F_{(1,80)}$ = 7.831, P < 0.001). Also, a species effect was detected ($F_{(9,80)}$ = 418.296, P < 0.001), which indicate that *rETRmax* of *Kappaphycus* sp. significantly higher and more sensitive, compare to *E. denticulatum.*

Regarding the mean values for PAR_{sat}, which indicates the value of PAR when rETR began to saturate, they increased with increasing temperature (Fig. 4.5.e). Temperature were significant factors in the differences determined for PAR_{sat} ($F_{(9,80)}$ =

19.951, $P < 0.001$), and there were interactions among temperature and species ($F_{(9,80)}$ = 2.207, $P < 0.05$), however, there were no significant effect of these species on PAR_{sat} $F_{(1,80)}$ = 3.462, P = 0.068). A model which describe a relation between temperature and PAR_{sat} of *E. denticulatum* and *Kappaphycus* sp. were linier ($F_{(2,39)}$ = 29.482, P <0.001 and ($F_{(2,39)}$ = 116.522, P <0.001), respectively. ($F_{(2,39)}$ = 116.522, P <0.001) for *Kappaphycus* sp. The relation between temperature (t) and PAR_{sat} of *E. denticulatum* and *Kappaphycus* sp. could be described by equation: PAR_{sat} = 49.3 + 3.199 t (R^2 = 0.437) and PAR_{sat} = 24.364 + 4.461 t (R^2 = 0.754), respectively. Their PAR_{sat} values were showed in Table 4.2.

The PAR where $rETR_{max}$ was observed (at PAR_{opt}), they increased with increasing temperature and reached their peak at higher temperatures (Fig. 4.5.f), with a significant temperature effect ($F_{(9,80)}$ = 25.049, $P < 0.001$) and a significant species effect ($F_{(1,80)}$ = 20.920, P < 0.001). There were also significant interactions describing the relationship between species and temperature ($F_{(1,24)}$ = 2.980, $P < 0.01$), where PAR_{opt} for *Kappaphycus* sp. was generally higher and more sensitive to temperature, compared to *E. denticulatum*. A model which describe a relation between temperature and PAR_{opt} of *E. denticulatum* and *Kappaphycus* sp. were quadratic ($F_{(2,39)}$ = 25.169, $P <$ 0.001 and $F_{(2,39)}$ = 48.174, $P < 0.001$, respectively). The relation between temperature

Table 4.2. Estimated saturating irradiance (PAR_{sat}) and maximum irradiance (PAR_{opt}) at some temperature levels of *E. denticulatum* and *Kappaphycus* sp. based on curve model estimation

Temperature	*E. spinosum*		*Kappaphycus* sp.	
(°C)	PAR_{sat}	PAR_{opt}	PAR_{sat}	PAR_{opt}
16	100.5	369.1	95.7	379.4
18	106.9	432.4	104.7	462.1
20	113.3	486.4	113.6	530.8
22	119.7	531.2	122.5	585.5
24	126.1	566.7	131.4	626.2
26	132.5	593.1	140.4	652.8
28	138.9	610.2	149.3	665.5
30	145.3	618.2	158.2	664.2
32	151.7	616.9	167.1	648.8
34	158.1	606.4	176.0	619.5

Note: The unit of PAR_{sat} and PAR_{opt} was μmol photons m^{-2} s^{-1}

PAR_{sat} = 49.3 + 3.199 t (R^2 = 0.437) for *E. denticulatum*

PAR_{sat} = 24.364 + 4.461 t (R^2 = 0.754) for *Kappaphycus* sp.

PAR_{opt} = -468.441 + 70.780 t – 1.152 t^2 (R^2 = 0.576) for *E. denticulatum*

PAR_{opt} = -786.596 + 100.889 t – 1.751 t^2 (R^2 = 0.723) for *Kappaphycus* sp.

(*t*) and PAR_{opt} of *E. denticulatum* and *Kappaphycus* sp. could be described by equation:

PAR_{opt} = -468.441 + 70.780 t – 1.152 t^2 (R^2 = 0.576) and PAR_{opt} = -786.596 + 100.889 t – 1.751 t^2 (R^2 = 0.723), respectively, and their values were showed in Table 4.2.

DISCUSSION

Irradiance against the depth decreased with increasing the depth (Lobban and Harrison, 1997). Relatively, the irradiance levels recorded at 300 m, 700 m and 1000 m from shore line have same trend (Fig. 4.2). In the specimen collecting area, the farmers cultivate *E. denticulatum and Kappaphycus* sp. in depth 1 to 2 m from the sea surface, indicating that they cultivate the specimen at irradiance ranged from 602 to 1177 μmol photons m^{-2} s^{-1} in the noon time. In shallow water, scattering and absorption of light are increased by the presence of much silt and numerous phytoplankton, and same amount of light may not reach 20 m (Lalli and Parsons, 1997). The farming area was surrounded by corral ref at approximately 1000 m from shore line, this condition can reduce the turbidity caused by wave effect on the sea bottom (Anthony *et al*., 2004).

In our study, the initial slope (α) of *E. denticulatum* and *Kappaphycus* sp. showed higher values at temperatures from 22 to 30 ˚C (Fig. 4.5.b). Meanwhile, the photoinhibition coefficient (β) of the two species decreased from low temperatures (Fig. 4.5.c), and $rETR_{max}$ increased from low temperatures to a peak between 30 to 32 °C (Fig. 4.5.d). These results suggest that the optimal temperatures of these two species is most likely with the range of 22 to 32 °C, and corresponds well to surface temperature recorded in Indonesian ocean (Sugiarto *et al*., 1976; Nonji, 1993; Amin, 2008). My

finding showed the range of optimal temperature for estimated parameters. 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, especially values of α and $rETR_{max}$. This reveals that the optimal temperature range for *E. denticulatum* is 22.7 to 31.5 °C and for *Kappaphycus* sp. it is 21.6 to 32.6 °C. Base on dissolved oxygen evolution, Wobeser *et al*., (2001) found that maximum photosynthesis (P_{max}) and respiration of *Kappaphycus alvarezii* (red and green morphotypes from Philippine) reach maximum value at temperature 30 °C. Ohno *et al*., (1994) cultured *K. alvarezii* in the subtropical water of Japan and found the highest growth rates occurred at temperatures of 25-28 °C. As a comparison, *in vitro* growth of sub-tropical macroalgae, *M. papulosa*, was optimum at temperature ranging from 20 to 24°C (Lideman *et al*., 2011), and at temperature 26 – 28 C, *Laurencia brongniartii* reach their highest growth and photosynthetic activity (Nishisaha *et al*., 2004a).

The maximum rETR in the absence of photoinhibition (γ) of these species increased with increasing of temperatures (Fig. 4.5.a), while, a previous study on photosynthetic of *Meristotheca coacta* and *M. papulosa* (chapter 3) showed that γ value

was monotonic linier from temperature 8 to 34 °C. This study finding may relate to the photoinhibition effect which was not clear for PAR less than 1000 μmol photons m^{-2} s^{-1} and it was lower for *E. denticulatum* and *Kappaphycus* sp. (Fig. 4.5.c) comparing to photoinihibition affecting the photosynthetic activities of *M. coacta* and *M. papulosa*. In general, the photoinhibition was not clear occurred at optimal temperature, this phenomenon indicate that these macroalgae need a time to have a photo-inhibition effect at high light intensities (Kirk, 2011). Harris and Piccinin (1977) recorded a decline in photosynthetic activity began after about 10 min of exposure because of photoinhibition. Furthermore, photoinhibition of *P-I* curve of *Kappaphycus alvarezii* obtained by dissolved oxygen method also did not clear at 20 °C until irradiance 650 μmol photons m^{-2} s^{-1} (Wobeser *et al*., 2001). It should be noted that photoinhibition data showed the high standard error and low value when examined RLCs until 1000μmol photons m^{-2} s^{-1}, indicating that these macroalgae could adapted to the light in their habitat, however, examining the photoinhibition more than 1000 μmol photons m^{-2} s^{-1} is necessary especially for tropical macroalgae.

The initial slope (α) of *Kappaphycus* sp. was always higher than that of *E. denticulatum* at each temperature condition examined (Fig. 4.5.b). However, the PAR_{sat} value was not different each other (Fig. 4.5.e), suggesting that these species can

photosynthesize under same levels of PAR to saturate their maximum photosynthetic activities. PAR_{opt} describes a PAR level which is photosynthetic activities reach their maximum capacity (Sigee, 2005), our finding showed that PAR_{opt} value of *Kappaphycus* sp. was higher than *E. denticulatum*, indicating that *E. denticulatum* need lower PAR to reach their maximal photosynthetic capacity and an evidence showed that sometimes upper fronds of *E. denticulatum* which exposure directly to the sun become colorless (lose their color). We guess that the colorless because the PAR was higher than PAR for their maximal photosynthetic capacity.

PAR_{sat} value of *E. denticulatum* and *Kappaphycus* sp. (Fig. 4.5.e) measured in this study increased with increasing of water temperature (Collins and Boylen, 1982; Palmisano *et al*., 1987; Henley, 1992, 1993). As a sublittoral algal species, PAR_{sat} of *E. denticulatum* and *Kappaphycus* sp. were inside a range of Lüning (1981), which suggested that in the upper and mid-sublittoral, algae species only require light ranging from 150 to 250 μmol photons m^{-2} s^{-1}. *E. denticulatum* and *Kappaphycus* sp. are generally cultured at the depth from 1 to 2 m deep. In this study, the mean values for the PAR_{sat} estimated at the examined temperatures (Fig. 4.5.e, Table 4.2) were lower than estimated maximum PAR (Fig. 4.2, Table 4.1). 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).

The response of these organisms over the range of experimental temperatures can be predicted by modeling the *P-I* curve and the relationship between the estimated parameters and temperature. 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. Temperatures ranges that optimal for maximum photosynthetic activity were provided in this study. 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. Models describing the rETR performance of *E. denticulatum* and *Kappaphycus* sp. 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.

CHAPTER 5: SUMMARY AND CONCLUSION

Three species of Solieriaceae (Rhodophyta), *Betaphycus gelatinus*, *Eucheuma serra* and *Meristotheca papulosa* were used to examine their *in vitro* growth rate and their photosynthetic activity at some temperatures in chapter 2. They are economically important species found in subtropical and tropical seawaters and they are well present in the coastal area of Okinawa and Kyushu Islands of Japan. Three genera, *Euchauma*, *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). In fact, Japan has been one of the largest *carrageenan* importing countries in the world (Ohno *et al*., 1994), the warm seawater area which is in southern part of Japan could be used in order to cultivate carrageenophytes as a source of *carrageenan*. Although a trial experiment of *M. papulosa* for their aquaculture has started by the Government of Ehime Prefecture (unpublished), but some cultivation factors is still not clear especially about optimum temperatures, irradiance conditions and good seeding, anyway there was no report on farming of *B. gelatinus* and *E. serra* in 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 affected 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 μmol photons m^{-2} s^{-1} at 24°C influenced photosynthetic rates. Photosynthetic parameters of these specimens were calculated by fitting photosynthesis versus irradiance curve (*P-I* curve) to non linear model of the formula: $P_{net} = (P_{max} \times \tanh [\alpha/P_{max} \times I]) + R_d$. Optimal relative growth rates of *B. gelatinus* and *E. serra* under 90 μmol photons m^{-2} s^{-1} were occurred at temperature ranged from 24°C to 28°C, while for *M. papulosa* it ranged from 20°C to 24°C. Maximum photosynthetic rates (P_{max}) for *B. gelatinus*, *E. serra* and *M. papulosa* were 135.0, 65.0 and 52.4 μg O_2 (mg chl-a)$^{-1}$ min^{-1}, respectively and saturating irradiance were 94.9, 69.4 and 35.4 μ mol photons m^{-2} s^{-1}, respectively. These characteristic results of temperatures and light were closely related to their depth of the habitat and local distribution in Southern Japan. This study provides information which is useful to design and manage their mass-cultivation systems. Based on the results, the cultivation of *B. gelatinus* can be conducted throughout a year in Ishigaki Is. (Okinawa Prefecture), while for *M. papulosa* and *E. serra* cultivation is possible from May to July in southern part of Kyushu Is. (Kagoshima and Miyazaki Prefecture).

In chapter 3, 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 physiological aspect, with ultimate goal of designing a cultivation system and elucidating their future distribution. These species grow well in coastal ecosystems of Japan, especially in Southern Japan (Yoshida and Yoshinaga, 2010). Additionally, *carrageenan* has been isolated from some species of *Meristotheca*, such as *M. papulosa* (Usov *et al*., 2001) and *M. senegalensis* (Fostier *et al*., 1992). In this chapter, I focused on examining the fluorescence of chlorophyll in order to describe the influence of temperatures and light (photosynthetic active radiation, PAR) on the photosynthetic activity. Unlike in chapter 2 that used dissolved oxygen probe, we used the pulse amplitude modulated (PAM)-chlorophyll fluorometry in chapter 3. This equipment has been used for some seagrass as well as terrestrial plants (Beer *et al.*, 1998; Beer and Björk, 2000; Kuster *et al*., 2007; Aldea *et al*., 2006; Ralph *et al*., 1998, 2006) and can be applied quickly and efficiently in analyzing photosynthetic response from the intact plants and also application for the photosynthetic responses of some seaweed has also been reported in recent years (Gevaert *et al*., 2002). An Imaging-PAM (Heinz Walz GmbH, Germany) 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 μmol photons m^{-2} 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 formula: $rETR = \gamma(1-\exp(-\alpha \cdot PAR/\gamma)) \cdot (\exp(-\beta \cdot PAR/\gamma))$ using a two-level hierarchical Bayesian model. Underwater PAR was measured near the study sites in order to describe the relation between results and characteristics of light in the habitat. The results suggest that the optimal temperatures of these two species is most likely with the range of 18 to 28 °C, and correspond well to an earlier study of *M. papulosa* that examined dissolved oxygen production and respiration rates (Lideman *et al*., 2011), and they are also in the range of water temperatures observed in their natural habitat. 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 (Lobban and Harrison, 1997). The relationship between saturating irradiance (PAR_{sat}) and temperature (t) were PAR_{sat} = -13.2050 + 4.5362t for *M. coacta* and PAR_{sat} = -40.0674 + 8.3777t for *M. papulosa*. PAR_{sat} and PAR_{opt} value of *M. coacta* and *M. papulosa* measured in this study increased with increasing of water temperature (Collins and Boylen, 1982; Palmisano *et al*., 1987; Henley, 1992, 1993). I 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 μmol photons m^{-2} s^{-1}. *M. coacta* and *M. papulosa* can be considered well-adapted to the current natural light and temperature conditions of southern Kyushu, Japan, changing water temperatures may have a drastic effect on their distribution. Based on our results I suggest that both of the species can be successfully cultivated from April to August in Kyusyu Island of Japan (Kumamoto, Miyazaki and Kagoshima Prefecture). Finding in this study should be useful to the design and manage mariculture programs and tank cultivation systems.

I used the tropical Red Algae, *Eucheuma denticulatum and Kappaphycus* sp. (Sumba strain), in order to examine their photosynthetic activity affected by temperature and light in chapter 4. They are cultivated largely in tropical area especially in South East Asia, particularly in Indonesia and the Philippines (Bixler and Porse, 2010), and Indonesia has become the major exporter of *Eucheuma* raw materials supplying the foreign demand for iota-carrageenan (Adnan and Porse, 1987). Mariculture of the genera *Kappaphycus* and *Eucheuma* supplies most of the *carrageenan* and carrageenan-like products (Dawes *et al*., 1994). Some strains under the name of

Kappaphycus alvarezii or related species are cultured in Indonesia such as *Sakol, Tambalang, cottonii* and *Sumba*. *Sumba* strain (*Kappaphycus* sp.) is predicted come from Sumba Island in Indonesia, while *Sakol* and *Tambalang* strain are transported from Philippines in 1990s. In Indonesia, the *Sumba* Strain, a strain of *Kappaphycus*, and *E. denticulatum* are known as the prominent commodity cultured, however, there is no sufficient knowledge for physiological aspect that could improve in cultivation system for Indonesian *Eucheuma/Kappaphycus* aquaculture. Especially, optimum temperature and light for the photosynthesis have not been clarified. Cultured specimens of *Eucheuma denticulatum* and *Kappaphycus* sp. (Sumba starin) were collected at farming area in Takalar, South Sulawesi, Indonesia. Rapid light curves (RLCs) were generated by running the standard algorithm of the pulse amplitude modulated (PAM)-chlorophyll fluorometer (Diving-PAM, Heinz Walz GmbH, Germany) using an incremental sequence of actinic illumination periods, with light intensities increasing in 9 steps from 0 to 1,000 μmol photons $m^{-2}s^{-1}$ of PAR. Using similar procedure in chapter 3, I calculated the photosynthetic parameters and derived the model to estimate the relation among the temperatures and photosynthetic parameters. Therefore, based on the values of each parameter estimated, I suggest that the range around 22 to 32 °C can be regarded as the optimum temperatures for the electron transport of two species. These

temperatures correspond well with the surface temperature in the mariculture farm as well as those recorded in Indonesian waters (Sugiarto *et al*., 1976; Nonji, 1993; Amin, 2008). Similar with result in chapter 3, PAR_{sat} value of *E. denticulatum* and *Kappaphycus* sp. measured in this study increased with increasing of water temperature (Collins and Boylen, 1982; Palmisano *et al*., 1987; Henley 1992, 1993). As a sublittoral algal species, PAR_{sat} of *E. denticulatum* and *Kappaphycus* sp. were inside a range of Lüning (1981), which suggested that in the upper and mid-sublittoral, algae species only require light ranging from 150 to 250 μmol photons m^{-2} s^{-1}. *E. denticulatum* and *Kappaphycus* sp. are generally cultured at the depth from 1 to 2 m deep, indicating that the farmer cultivate the specimens at irradiance ranged from 602 to 1177 μmol photons m^{-2} s^{-1} in the noon time. In this study, the mean values for the PAR_{sat} estimated at the examined temperatures were lower than estimated maximum PAR. Indeed, saturating irradiances show some correlation with habitat, but generally they are low compared to full sun (Reiskind *et al*., 1989). The results of this study can be used as the basic information to develop cultivation system that will maximize production and minimize costs at the commercial scale in Indonesia.

In conclusion, some endemic species could be cultivated in warm water of Southern Japan, however we need more experiment and practice in the field to apply the

findings in this study. The estimates of optimal temperature of all species used in this study have close correlations with the temperature of their habitat. To saturate their maximum photosynthesis, these species required light intensity that increased with increasing the temperatures. Models describing the rETR performance 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.

REFERENCES

Adnan, H and H. Porse (1987) Culture of *Eucheuma cottonii* and *Eucheuma spinosum* in Indonesia. *Hydrobiologia,* **151/152** (Dev. Hydrobiol. 41), 355-358.

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

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

Anderson, R. (2005) *Algal Culturing Techniques*, Elsevier Academic Press, Burlington, USA, 596 pp.

Anthony, K. R. N., P. V. Ridd, A. R. Orpin, P. Larcombe and J. Lough (2004) Temporal variation of light availability in coastal benthic habitats. *Limnol. Oceanogr*., **49**, 2201–11.

Barsanti, L. and P. Gualtieri (2006) *Algae: Anatomy, Biochemistry and Biotechnology*. New York: CRC Press.

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

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

Bhujel, R. C. (2008) Statistics for Aquaculture. Wiley-Blackwell, Iowa, USA, 222p.

Bixler, H.J. and H. Porse (2010) A decade of change in seaweed hydrocolloids industry. *J. Appl. Phyco*l. DOI 10.1007/s10811-010-9529-3.

Chapman, V. J. (1970) Seaweeds and Their Uses, 2nd edn. Methuen, London.

Collins, C. D. and C. W. Boylen (1982) Physiological responses of *Anabaena variabilis* (Cyanophyceae) to instantaneous exposure to various combinations of light intensity and temperature. *J. Phycol*. **18**: 206-211.

Dawes C. J., A. O. Lluisma and G. C. Trono (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.

Dawes, C. J., A. C. Mathieson and D. P. Cheney (1974) Ecological studies of Floridian *Eucheuma* (Rhodophyta, Gigartinales). I. Seasonal growth and reproduction. *Bull. Mar. Sci.*, **24**, 235-273.

Dawes, C. J., J. Orduna-Rojas and D. Robeldo (1999) Response of the tropical red seaweed *Gracilaria cornea* to temperature, salinity and irradiance. *J. Appl. Pycol*. **10**: 419-425.

Dominigues, C. M., J. A. Church, N. J. White, P.J. Gleckler, S. E. Wijffels, P. M. Barker and J. F. Dunn (2008) Improved estimates of upper-ocean warming and multi-decadal sea-level rise. *Nature,* **453**: 1090-1093.

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.

Faye, E. J., K. Kogame, S. Shimada, S. Kawaguchi and M. Masuda (2007) Taxonomic features of the red alga *Meristotheca coacta* (Solieriaceae, Gigartinales) from Japan. *Phycol. Res.* **55**: 150-158.

Faye, E. J., K. Kogame, S. Shimada, S. Kawaguchi and M. Masuda (2008) New red alga *Meristotheca imbricata* (Solieriaceae, Gigartinales) from Japan. *Phycol. Res.* **56**: 115-126.

Fostier, A. H., J. M. Kornprobst and G. Combaur (1992) Chemical composition and reological properties of carrageenan from two Senegalense Solieriaceae, *Anatheca montagnei* Schmitz and *Meristotheca senegalense* Fielmann. *Bot. Mar.* **35**: 351-355.

Gerung, G. S. and M. Ohno (1997) Growth rates of *Euchema 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.

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

Glenn, E. P. and M. Doty (1990) Growth of the seaweeds *Kappaphycus alvarezii*, *K. striatum* and *Eucheuma denticulatum* as affected by environment in Hawaii. *Aquaculture*, **84**, 245-255.

Guiry, M. D. in Guiry, M. D. and G. M. Guiry (2011) *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org. Accessed on 25 September 2011.

Harris, G. P. and B. B. Piccinin (1977) Photosynthesis by natural phytoplankton populations. *Arch. Hydrobiol.*, **80**, 405-57.

Henley, W. J. (1992) Growth and photosynthesis of *Ulva rotundata* (Chlorophyta) as a function of temperature and square wave irradiance in indoor culture. *J. Phycol*. **28**: 625-634.

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.

Herr, D. and G. R. Galland (2009) *The Ocean and climate change. Tolls and guidelines for action*. IUCN, Gland, Switzerland. 72 pp.

Huang, S. F. (2000) *Seaweeds of Northeastern Taiwan*. pp. xii, 1-233. Taipei: National Taiwan Museum.

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.

JMA. (2011a) Long-term trends in sea surface temperature (Northern Higashishinakai, Japan). Available at: http://www.data.kishou.go.jp/kaiyou/db/nagasaki/nagasaki_warm/nagasaki_warm_areaC.html#title. Accessed 28 June 2011.

JMA. (2011b) Sea surface temperature in Japan. Available at: http://www.data.kishou.go.jp/kaiyou/db/kaikyo/knowledge/sst.html. Accessed 20 June 2011.

Japan Meteorology Agency (2010). Available at: 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 *Eucheuma 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 *Eucheuma*. *J. Appl. Phycol.* **11**, 149-156.

Kirk, J. T. O. (2011) The nature of the underwater light field. In: Light and Photossynthesis in Aquatic Ecosystem. 3nd ed. (page: 112-128). New York: Cambridge Univ. Press.

Kraft, G. T., L. M. Liao, A. J. K. Millar, E. G. G. Coppejans, M. H. Hommersand and F. D. Wilson (1999) Marine benthic red algae (Rhodophyta) from Bulusan, Sorsogon Province, Southern Luzon, Philippines. *The Philippine Scientist* **36**: 1-50

Kuster, A., K. Pohl and R. Altenburger (2007) A Fluorescence-Based Bioassay for Aquatic Macrophytes and its Suitability for Effect Analysis of Non-Photosystem II Inhibitors. *Env. Sci. Pollut. Res*.**14** (6): 377–383

Lalli, C. M. and T. R. Parsons (1997) Biological Oceanography An Introduction. 314 pp: Elsevier. Vancouver, Canada.

Lee, Y. and S. Kang (2001) *A catalogue of the seaweeds in Korea*. pp. 8, 1-662. Jeju: Cheju National University Press.

Lideman, G. N. Nishihara, T. Noro and R. Terada (2011) *In Vitro* Growth and Photosynthesis of Three Edible Seaweeds, *Betaphycus gelatinus*, *Eucheuma serra* and *Meristotheca papulosa* (Solieriaceae, Rhodophyta). *Aquaculture Sci.* **59** (4): 563-571.

Lobban, C. S. and P. J. Harrison (1997) *Seaweeds Ecology and Physiology*, Cambridge University Press, Cambridge, UK, 384 pp.

Lüning, K. (1981) Light. In: Lobban, C. S. and M. J. Wynne (eds.). *The Biology of Seaweeds* (pp. 326-55). Blackwell Scientific. Oxford.

Luxton, D.M., 1993. Aspects of the farming and processing of Kppaphycus and Eucheuma in Indonesia. *Hydrobiologia*, **260-261** (1), 365-371.

Maegawa, M., M. Kunieda 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.

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.

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

Muraoka, D., H. Yamamoto, H. Yasui and R. Terada (1998) Formation of wound tissue of *Gracilaria chorda* Holmes (Gracilaceae) 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. and R. Terada (2010) Spatial variations in nutrient supply to the red algae *Eucheuma serra* (J. Agardh) J. Agardh. *Phycol. Res.*, **58**, 29-34.

Nishihara, G. N., R. Terada and T. Noro (2004a) Photosynthesis and growth rates of *Laurencia brongiartii* 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.

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

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.

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

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

Ohno, M., M. Yano, M. Hiraoka, N. Oka and M. Taniguchi (2002) Tank culture of *Echeuma serra* and *Meristotheca papulosa* using with deep sea water. *Bull. Mar. Sci. Fish., Kochi Univ.*, **20**, 35-40 (in Japanese).

Palmisano, A. C., J. B. SooHoo and C. W. Sullivan (1987) Effects of four environmental variables on photosynthesis-irradiance relationships in Antarctic sea-ice microalgae. *Mar. Biol*. **94**: 299-306.

Parker, H. S. (1974) The culture of the red algal genus Eucheuma 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.

Platt, T., C. L. Gallegos and W. G. Harrison (1980) Photoinhibition of photosynthesis in natural assemblages of marine phytoplankton. *J. Mar. Res*. **38**: 687-701.

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.

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., A, Gademann, and W. C. Dennison (1998) In situ seagrass photosynthesis measured using a submersible, pulse-amplitude modulated fluorometer. *Marine Biology*, **32**: 367-373

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

Reiskind, J. B., S. Beer and G. Bowes (1989) Photosynthesis, photorespiration and ecophysiological interaction in marine macroalgae. *Aquat. Bot*. **34**: 131-152.

Santos, G. A. (1989) Carrageenans of species of Eucheuma J. Agardh and Kappaphycus Doty (Solieriaceae, Rhodophyta). *Aquat. Bot.*, **36**, 55-67.

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

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).

Sigee, D.C. (2005) Freshwater Microbiology. John Wiley & Sons Ltd. England.

Soegiarto A., Birowo S, and Sukarno (1976) Map of Indonesian Ocean. Indonesian Science Institute.

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.

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

Trono, G. C. Jr. (1993) *Eucheuma* 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.

Tseng, C. K in Loban C. S and M. J. Wynne (1981) The Biology of Seaweeds. Blackwell Scientific Publications. Oxford, London, Edinburgh, Boston and Melbourne.

Tsuchiya, Y., Y. Sakaguchi and R. Terada (2011) Phenology and environmental characteristics of four *Sargassum* species (Fucales): *S. piluliferum, S. patens, S. crispifolium*, and *S. alternato-pinnatum* from Sakurajima, Kagoshima Bay, southern Japan. *Jpn. J. Phycol*. **59**: 1-8 (in Japanese).

Usov, A. I., A. V Podkorytova, H. D. Yoon (2001) Structure of a sulfated from *Meristotheca papulosa* (Rhodophyta, Solieriaceae) from South Korea. *Abstract 2.2.8 XVIIth Int. Seaweed Symp*. South Africa.

Waaland, J. R in Loban C. S and M. J. Wynne (1981) The Biology of Seaweeds. Blackwell Scientific Publications. Oxford, London, Edinburgh, Boston and Melbourne.

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

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

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.

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