

# ホソメコンブ *Laminaria religiosa* Miyabe 配偶体の光- 光合成の温度依存性

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## Temperature dependence of photosynthesis-irradiation (P-I) relationship of gametophytes of *Laminaria religiosa* Miyabe

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Photosynthetic characteristics of gametophytes of *Laminaria religiosa*, an endemic Laminariales of northern Japan, were investigated. Photosynthetic activity (P) was measured for a range of irradiance (I) between 0 and 942  $\mu\text{Em}^{-2}\text{s}^{-1}$ , and in a temperature range between 2 and 30°C. The P-I curve was approximated by a hyperbolic tangent function;  $P = P_{\text{max}} \times \tanh(I/I_K) - P_0$ . The highest  $P_{\text{max}}$  was 10.9 mmol O<sub>2</sub> (g chl a)<sup>-1</sup>min<sup>-1</sup> at 20°C.  $I_K$  was higher than that reported for other Laminariales. Temperature optimum for photosynthesis was 15 to 20°C when  $I > 120 \mu\text{E m}^{-2}\text{s}^{-1}$ , but there was no apparent optimum peak when  $I < 120 \mu\text{E m}^{-2}\text{s}^{-1}$ . It is suggested that the gametophytes of *L. religiosa* adapt photosynthetic characteristics to its ecological and environmental conditions.

### Introduction

Laminariales are characterized by a complex life cycle with alteration of macroscopic sporophytes and microscopic gametophytes. Generally, geographical distribution of seaweeds is governed by the tolerance at the hardest life-cycle phase, often the microthallus phase, and by the reproductive ability in either phase (Lüning, 1990). The alteration of both phases enables seaweeds to survive and reproduce in a range of conditions in various waters. Physiological characteristics of the two phases are probably different to cope with seasonal changes of environmental conditions (Lee and Brinkhuis, 1986).

*Laminaria religiosa*, endemic to southwestern Hokkaido and the Pacific coasts of northern Honshu, is the only Laminariales in Japan whose sporophytes are mainly seasonal annuals (Kawashima, 1993). Most sporophytes disappear during autumn and early winter. Gametophytes are the only form to survive during that period (Hasegawa *et al.*, 1963), suggesting that gametophytes are more adaptive to environmental conditions during autumn and early winter than sporophytes. Physiological characteristics of gametophytes could play an important role in maintaining its populations (Abe *et al.*, 1990). Although sporophytes of *L. religiosa* has been studied on their ecological and physiological characteristics (Hasegawa *et al.*, 1963; Matsuyama *et al.*, 1982; Matsuyama, 1985), there is no report on the physiological characteristics of the gametophytes.

The objective of the present paper is to describe temperature and light effects on the photosynthesis of gametophytes of the alga, and to discuss their physiological adaptation to environments.

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### Materials and methods

Fertile sporophytes were collected in September at Yoichi Bay, Hokkaido. Zoospores were obtained by immersing mature sorus parts, which had been kept in moist air overnight, in glass fiber (GF/C)-filtered seawater. Released zoospores were pipetted into culturing chambers (Multiple Well Plate, Corning Co.) filled with 3ml of artificial seawater (iron-free ASP<sub>12</sub>; Motomara and Sakai, 1984). Iron was omitted because, in the original ASP<sub>12</sub> medium, gametophytes become fertile and the harvest of pure gametophytes was not possible. Gametophytes were cultured at 10°C under fluorescent lamps (irradiance ca. 33 $\mu\text{Em}^{-2}\text{s}^{-1}$ , light : dark cycle = 14h : 10h). The culture medium was exchanged every one or two months. Gametophytes grew vegetatively and formed filamentous plants. Gametophytes were harvested when their diameters became larger than ca. 1mm, and transferred to 300ml flasks with 100ml of enriched seawater (PES; Provasoli, 1968). Gametophytes were cultured under the same conditions with shaking.

Before photosynthesis by gametophytes was measured, gametophytic clumps were disintegrated by using a Waring blender (10 seconds at ca. 10000rpm in filtered seawater), because irradiance at the surface and the inside of the clump would be different when whole clumps were used for the measurement. Gametophytes between 265 $\mu\text{m}$  and 100 $\mu\text{m}$  in size were collected by filtering through a pair of plankton nets. The gametophytes were suspended in the filtered seawater. The sample was preincubated in the same conditions as above overnight.

Photosynthetic activity was measured by using a Clark type oxygen electrode (Rank Bros. Co.). Four milliliters of the gametophyte suspension was placed in a measuring chamber. Concentration of the gametophyte was 0.70 $\mu\text{g chl ml}^{-1}$ . Light of a tungsten-halogen lamp (JCR15V150W, 3100°K, Hayashi Watch Works Co.) with a heat-cutting filter (50% cut at 730nm) was guided with a pair of light-guiding glass fibers set at opposite sides of the chamber to make the light conditions even. Irradiance in the chamber was measured with a hand-built light meter, which was calibrated with a quantum meter (Biospherical Instrument). At the beginning of each measurement, air was bubbled for ca. 10min to make the medium air-saturated. Changes in oxygen concentration at six different irradiances (0, 16, 52, 117, 419, and 914 $\mu\text{Em}^{-2}\text{s}^{-1}$ ) were measured consecutively from low to high irradiance. The measuring time span was adjusted so that the final oxygen concentration did not exceed 110% of the air-saturation. Measurements were conducted at seven different temperatures (2, 5, 10, 15, 20, 25, and 30°C).

A hyperbolic tangent function (Gallegos and Platt, 1981) was used to express a photosynthesis-irradiance (P-I) relationship of the gametophytes:

$$P(I) = P_{\max} \times \tanh(I/I_K) - P_0 \quad (1),$$

Where P(I) denotes gross photosynthesis (oxygen evolution) at irradiance I; P<sub>max</sub>, maximum gross photosynthesis; I<sub>K</sub>, irradiance for photo synthesis saturation; P<sub>0</sub>, dark oxygen consumption (e. i. respiration). Parameters; P<sub>max</sub>, I<sub>K</sub>, and P<sub>0</sub>, were estimated by a least square method.

A chlorophyll a content was measured with a N, N-dimethylformamide extraction method (Suzuki and Ishimaru, 1990).

### Results and Discussion

Oxygen evolution increased with the increase of irradiance and was saturated when the

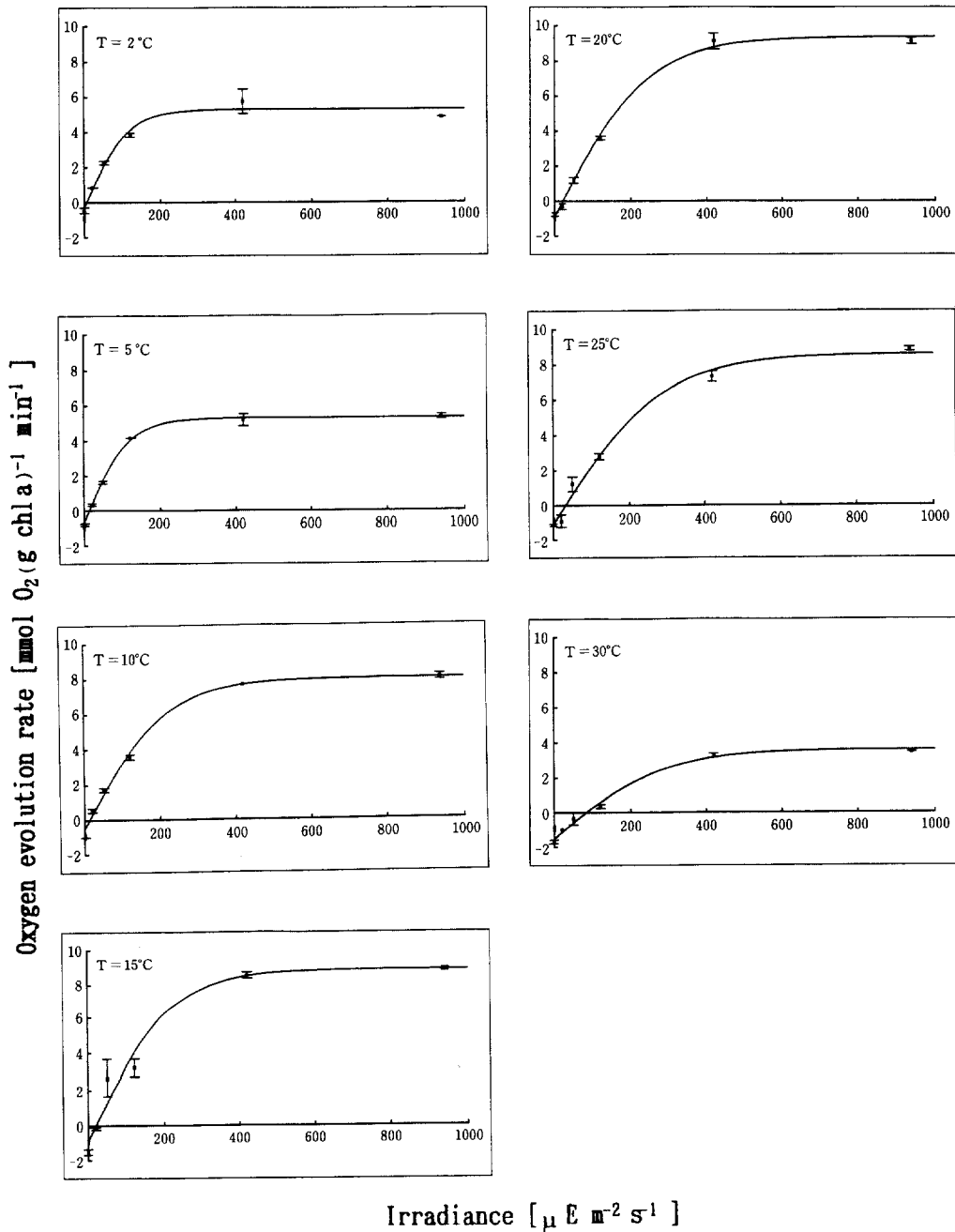


Fig. 1. Net photosynthesis (oxygen evolution) vs. irradiance relationship of gametophytes of *L. religiosa* at temperatures between 2 and 30°C. Dots show the observed rates with one standard deviation. Lines show the approximated hyperbolic tangent functions (1) fitted with a least square method.

irradiance was over  $400\mu\text{Em}^{-2}\text{s}^{-1}$  (Fig. 1). There was no apparent photoinhibition below  $942\mu\text{Em}^{-2}\text{s}^{-1}$ . The P-I relationship was closely approximated with a hyperbolic tangent function (Fig. 1 and Table 1). Matsuyama (1985) used a Michaelis-Menten type function to approximate the P-I curve of the sporophytes of *L. religiosa*. He calculated  $I_k$  of sporophytes of the alga to range from 103 to  $350\mu\text{Em}^{-2}\text{s}^{-1}$ . As he collected samples in the fields at different seasons in a year to measure the photosynthetic activity at the in situ temperature, it is not possible to directly compare photosynthetic characteristics in his report with those in the present study on laboratory-cultured gametophytes. However, there may not be a large difference between  $I_k$ , an index for the photosynthetic adaptation to environments, of sporophytes and that of gametophytes.

The temperature effect on photosynthesis in ranges of irradiance was clearly shown when the oxygen evolution rates calculated by using the parameters in Table 1 were plotted three-dimensionally (Fig. 2). Where irradiance was higher than ca.  $120\mu\text{Em}^{-2}\text{s}^{-1}$ , shown by a broken line in Figure 2, the photosynthesis curve had a ridge at 15 to 20°C. Below  $120\mu\text{Em}^{-2}\text{s}^{-1}$  of irradiance, the net photosynthesis was higher at lower temperature. This is due to a decrease in the respiration rate (the intercept at irradiance=0 in Fig. 2) at low temperature. Where an irradiance was low, gross photosynthesis ( $P(I)+P_0$ ) did not show an apparent change below 20°C. Compensation irradiance ( $I_c$ ), an intercept where oxygen evolution is zero in Fig. 2, increased from  $6.3\mu\text{Em}^{-2}\text{s}^{-1}$  to  $81\mu\text{Em}^{-2}\text{s}^{-1}$  with the increase of temperature from 2°C to 30°C (Table 1).

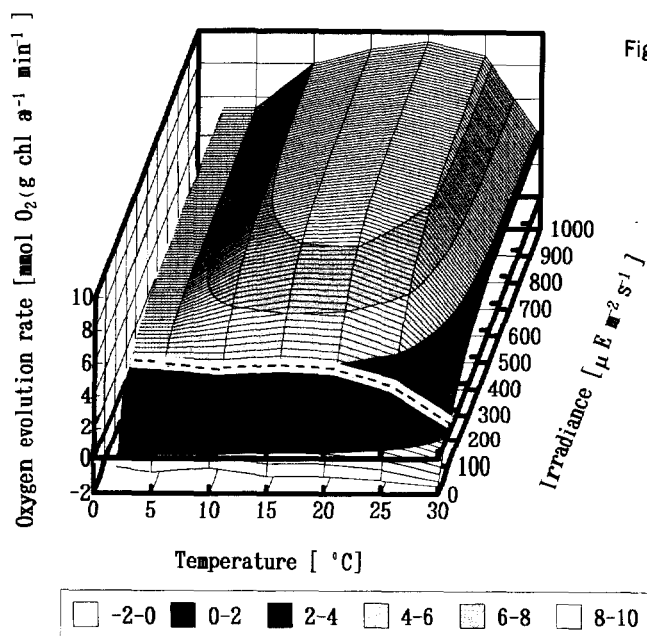


Fig. 2. Interrelationship of temperature and irradiance to net photosynthesis (oxygen evolution) of gametophytes of *L. religiosa*. Oxygen evolution rates are calculated with hyperbolic tangent functions shown in Fig. 1. The contour surface of the oxygen evolution rate is shaded every two units of  $\text{mmol O}_2 (\text{g chl a})^{-1}\text{min}^{-1}$ . A broken line indicates the oxygen evolution rate at  $I=120\mu\text{Em}^{-2}\text{s}^{-1}$ .

Gametophytes of Laminariales occurring in northern North Atlantic and eastern Pacific coasts have been studied on their responses to light and temperature (Lüning and Neushul, 1978; Lüning, 1980; and Gerard, 1990). By measuring the increase of the maximum diameter of female primary cells of gametophytes, Lüning and Neushul (1978) and Lüning (1980) reported that the rates of vegetative growth of *Laminaria digitata*, *L. saccharina*, *L. hyperborea*, *L. farlowii*, *L. dentigela*, *L.*

**Table 1** Photosynthetic characteristics of gametophytes of *Laminaria religiosa* calculated with a hyperbolic tangent function (1).

Temperature(°C)	2	5	10	15	20	25	30
P <sub>max</sub>	6	5.9	8.5	9.7	10.9	9.1	5.1
I <sub>k</sub>	126	109	213	212	266	249	275
P <sub>0</sub>	0.3	0.7	0.5	0.9	0.9	1.1	1.5
I <sub>c</sub>	6.3	13	13	20	22	30	81

Units : P<sub>max</sub> (maximum gross photosynthesis) and P<sub>0</sub> (respiration), mmol O<sub>2</sub> (g chl a)<sup>-1</sup>min<sup>-1</sup>; I<sub>k</sub> (irradiance of photosynthesis saturation) and I<sub>c</sub> (compensation irradiance), μEm<sup>-2</sup>s<sup>-1</sup>.

*sinclairii*, *Macrocystis pyrifera*, *M. integrifolia*, and *Egregia menziesii* were light-saturated at 20 μEm<sup>-2</sup> s<sup>-1</sup> or a little higher irradiance. Growth of male gametophytes was saturated at the same range. They suggested that saturation of photosynthesis took place at the same range. It was postulated that laminarian gametophytes are "characterized as extreme-shade plants" (Lüning and Neushul, 1978). From their figures, the irradiance of the growth saturation of female gametophytes is estimated to be about 20 μEm<sup>-2</sup>s<sup>-1</sup>. I<sub>k</sub> values for the gametophytes of *L. saccharina* are calculated to be 56-82 μEm<sup>-2</sup>s<sup>-1</sup> from the photosynthetic activity measurement by Gerard (1990). Those values are higher than those estimated by Lüning and Neushul(1978) and Lüning(1980). An experiment of photosynthesis of laminarian spores showed I<sub>k</sub> in a range of 41-77 μEm<sup>-2</sup>s<sup>-1</sup>, varying to species (Amsler and Neushul, 1991). It is not certain why the I<sub>k</sub> values are different in each report and our study. The photosynthetic characteristics may vary between the species and stages in the life cycle. Generally, laminarian sporophytes are perennial. Therefore, gametophytes have to survive under the canopy of their parent sporophytes. However, *L. religiosa* is a seasonal annual and seaweed beds are free of shading macrophytes after sporophytes have disintegrated. Thus, gametophytes can obtain full sun-light. Difference in the I<sub>k</sub> value may result from their specific types of life-cycles.

Another factor affecting the photosynthetic response of gametophytes would be acclimation. It is known that phytoplankton adapt physiologically to ambient light conditions by varying the amount of accessory pigments involved in harvesting photons (Valiela, 1984). Changes of photosynthetic characteristics responding to culturing conditions were observed on gametophytes of *L. saccharina* (Gerard, 1990).

Our work is the first report on the temperature dependence of the photosynthesis of gametophytes of *L. religiosa*. The optimum temperature for photosynthesis of gametophytes is between 15 and 20°C, when irradiance is higher than 120 μEm<sup>-2</sup>s<sup>-1</sup>. Okada and Sanbonsuga(1980) reported that female gametophytes of *L. religiosa* grew actively at up to 20°C. The temperature for optimum photosynthetic activity found in our work was in the range of the water temperature at Oshoro Marine Biological Station, ca. 7 km west of Yoichi Bay, in September to October (13-22°C, K. Shinta, personal communication), when the spores were actively released. Those observations suggest that gametophytes adapt to temperature variations in the habitat.

The P<sub>max</sub> of sporophytes of *L. religiosa* at 14.4°C in May, on the other hand, decreases by more than half at 20°C in July (Matsuyama, 1985). Gametophytes showed a different photosynthetic

response to temperature from sporophytes when irradiance was high (Fig. 2). In Matsuyama's experiments, the sporophytes that were collected from natural habitats in May and in July might have different physiological conditions. Those differences may result in different photosynthetic response of the sporophytes. To predict the performance of the gametophytes in the field, it is necessary to investigate the effects of not only temperature and light, but also other factors, such as nutrient conditions at the activity measurements and at preincubation.

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### ホソメコンブ *Laminaria religiosa* Miyabe 配偶体の光一光合成の温度依存性

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#### 摘 要

北海道西岸及び三陸沿岸に固有な褐藻、ホソメコンブの配偶体を培養し、その光合成に及ぼす光量子量と温度の影響を調べた。光量子量 ( $I$ ) は 0 から  $942 \mu\text{Em}^{-2}\text{s}^{-1}$ 、温度は 2 から  $30^\circ\text{C}$  の範囲において酸素電極法で光合成活性を測定した。配偶体の光合成—光量子量曲線は双曲線関数： $P(I) = P_{\max} \times \tanh(I/I_K) - P_0$  で近似でき、光合成は  $I = 200$  から  $400 \mu\text{Em}^{-2}\text{s}^{-1}$  で飽和し、飽和光合成活性 ( $P_{\max}$ ) は温度が 15 から  $20^\circ\text{C}$  で高く、最大  $10.9 \text{mmol O}_2 (\text{g chl a})^{-1}\text{min}^{-1}$  という値が得られた。しかし  $I < 120 \mu\text{Em}^{-2}\text{s}^{-1}$  の場合、光量子量が高い場合に比べ温度による影響が少なかった。また、低光量子量における光合成効率 (Photosynthetic efficiency,  $P_{\max}/I_K$ ) は、これまでコンブ科植物で報告されている値に比べ低いものとなった。これらの実験結果から、ホソメコンブ配偶体の光合成は分布海域の生態学的環境条件に適応した特性を持つと推定された。

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