Salinity and light effects on growth, photosynthesis, and respiration of *Grateloupia filicina* (Rhodophyta)

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Abstract

Effects of salinity and irradiance on growth, photosynthesis, and respiration of the commercially important red alga *Grateloupia filicina* (Lamouroux) C. Agardh were examined in laboratory cultures. *G. filicina* thalli showed various degrees of growth over a broad range of experimental conditions, with a maximum growth rate of 13% d⁻¹ observed at 20%e and 100 μmol photons m⁻² s⁻¹. Salinity also altered parameters of the photosynthesis-irradiance relationship. Maximum photosynthetic capacity \(P_{\text{max}}\) reached a high value of 109.2 μmol O₂ g⁻¹ fw⁻¹ h⁻¹ at 20%e but decreased to much lower values toward 0%e and 40%e. \(P_{\text{max}}\) at 0%e was only 15.3 μmol O₂ g⁻¹ fw⁻¹ h⁻¹. The respiration rate ranged between 3.0 and 9.6 μmol O₂ g⁻¹ fw⁻¹ h⁻¹. The respiration-to-\(P_{\text{max}}\) ratio \((R/P_{\text{max}}\) ratio\) showed an inverse relationship with the growth rate and had a minimum value of 0.07 at 20%e. These results suggest that salinity and irradiance need to be carefully controlled during mass cultivation of *G. filicina* in order to maximize growth and yield. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: Grateloupia filicina; Salinity; Irradiance; Growth; Photosynthesis; Respiration

1. Introduction

*Grateloupia filicina* (Lamouroux) C. Agardh, Rhodophyta, is an edible marine macroalgae. It is used as seaweed salad in Japan and is sold as a gourmet item in Taiwan.

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Carrageenan from *G. filicina* is also of commercial value. *G. filicina* has a carrageenan similar to the λ type. Its high content of 3,6-anhydrogalactose gives it a low viscosity (Zablackis and Perez, 1990), a property that is highly desirable in certain applications for the food industry (Guist, 1990).

*G. filicina* has been cultivated commercially in Japan (Tokuda et al., 1987). The developmental sequence is well studied, and much improvement has been made in culturing techniques (Chiang, 1993). In addition, a new technique in regeneration of new crusts for mass production has been developed (Migita, 1988; Iima et al., 1995). This technique eliminates the inconvenience of collecting blades from natural environments, where the supply is usually limited.

In cultivating seaweeds, it is important to maintain growth conditions in the optimal range to reduce the duration of cultivation and to ensure the greatest yield. For a species acclimated to a particular set of environmental conditions, the optimal growth is always achieved at a specific combination of salinity and irradiance (Brinkhuis et al., 1984; Hanisak, 1987; Lobban and Harrison, 1994). For large-scale outdoor operations, salinity and irradiance are two environmental factors that can be manipulated in inexpensive ways. Salinity in culture ponds can be decreased by diluting seawater with freshwater, and increased by solar evaporation. As for irradiance level, it can be adjusted by altering the depth of the crop or by providing shading for cultivation tanks. However, the optimal combination of salinity and irradiance varies greatly from one species to another, and needs to be determined specifically for the species of interest.

Salinity influences physiological events in seaweeds by changing the movement of water molecules and ions across the cell membrane (Kirst, 1989, 1995; Reed, 1990; Lobban and Harrison, 1994). The effects of salinity on the growth of *G. filicina* have been examined in an outdoor culture in Hawaii (Zablackis, 1987), where maximum growth occurred at 15%, but the thalli produced significantly more branches at salinities near 35%. However, how irradiance and salinity interact to regulate growth of *G. filicina* via changing photosynthesis and respiration rate is unknown. In the present study, the effects of irradiance and salinity on photosynthesis and respiration rates of *G. filicina* were examined, and a physiological basis was provided for altered growth rates under a gradient of these two environmental factors. Such information not only improves our understanding of the growth requirements of this commercial red alga, but will also be useful in selecting suitable sites and developing a proper management protocol for successful mass cultivations.

2. Materials and methods

2.1. Plant materials

Thalli used in the experiments were about 3 to 5 cm in length (ca. 4–5 month old after germination), and were raised from carpospores. The carpospores were collected from fertile gametophytes at Fulong village in northern Taiwan between 1995 and 1996, and utilized tissue culture techniques to obtain carpospores (Chiang, 1993).
2.2. Growth experiment

A total of eight salinities (5, 10, 15, 20, 25, 30, 35 and 40%e) and five irradiances (5, 30, 55, 100, and 135 μmol photons m$^{-2}$ s$^{-1}$ (400–700 nm)) were used to test the effects of both factors on the growth of *G. filicina*. Filtered (200 μm nylon mesh) natural seawater was used for incubation. Treatments with salinities less than 30%e were prepared by mixing natural seawater with fresh groundwater, while salinities greater than 30%e were achieved by adding synthetic sea salt (Reef Crystals, Instant Ocean, Mentor, OH). Concentrations of nitrate, nitrite, and phosphate were 28, 0.3, and 1.6 μM in the filtered seawater, and were 36, 0.7, and 3 μM in groundwater, respectively. In synthetic sea salt, all three nutrients were lower than 1 μM when prepared as a 30%e solution. As a result, nutrient levels were quite constant in various salinity treatments.

Illumination was provided by 40 W cool-white fluorescent lights with a 12:12 light: dark photoperiod. Various irradiance levels were created by adjusting the number of fluorescent tubes or by wrapping culture vessels with neutral density screens. Irradiance was measured using a 2π sensor (Li-Cor 190SA) connected to a datalogger (Li-Cor, LI-1000). All experiments were conducted in a walk-in incubator, with temperatures held constant at 23 ± 0.5°C.

Before each experiment, thalli were rinsed with sterile seawater, and cleaned with low-lint absorbent papers (Kimwipes, Kimberly-Clark) to remove epiphytes. Next, about 1 g of thalli was weighed, and then incubated in a beaker containing 1 l seawater. Duplicate beakers were set up for each light-salinity combination. Incubations lasted for 14 d, and the seawater used for incubation was changed every 2 d. After incubation, thalli were blotted dry, and weighed. The specific growth rate was then calculated according to the following equation (Brinkhuis, 1985):

\[
\text{Growth rate (} \% \text{ d}^{-1} \text{)} = \frac{1}{t} \left( \ln \frac{W_f}{W_i} \right) \times 100\%
\]

where $W_i$ and $W_f$ are the initial and final fresh weight (fw), respectively, and $t$ is the duration of the incubation period (d).

2.3. Photosynthesis and respiration

Change in oxygen concentration over a 6 h experimental period was used to measure the photosynthesis and respiration rates of *G. filicina*. Thalli of 0.5 ± 0.01 g were placed in 300 ml BOD bottles and were incubated at 23 ± 0.5°C. The photosynthesis–irradiance response of *G. filicina* was evaluated at five salinities (0, 10, 20, 30, and 40%e), and individual photosynthesis–irradiance curves were constructed using irradiance levels at 0, 0.6, 8, 38, 57, 80, and 124 μmol photons m$^{-2}$ s$^{-1}$, respectively. Different irradiances were created by adjusting the number of fluorescent tubes as well as the distance between the light source and BOD bottles. Duplicate bottles were used at each setting but triplicates were used for the dark treatment. In addition, two blank bottles containing seawater only were used as controls. To avoid transient responses resulting from sudden
salinity shifts, thalli were acclimated at the experimental salinity for 24 h prior to incubation.

Oxygen concentration was measured following the method described by Pai et al. (1993). The oxygen consumption rate in the dark bottles were used as the respiration rate, and the net photosynthetic rate was calculated as the difference in oxygen concentration in the light bottles between the onset and the end of incubation. The parameters $P_{\text{max}}$ and $I_k$ of the photosynthesis–irradiance curve were estimated by the relationship between net photosynthetic rate and irradiance using a nonlinear regression software (SigmaPlot, Jandel Scientific Software, San Rafael, CA) according to the following equation (Falkowski and Raven, 1997):

$$P = \frac{(P_{\text{max}} \times I)}{(I_k + I)} - R$$

where $P$ is the net photosynthetic rate in moles O$_2$ g fw$^{-1}$ h$^{-1}$; $I$ is irradiance; $P_{\text{max}}$ is the maximum photosynthetic capacity; $I_k$ is the half-saturation irradiance level; and $R$ is the respiration rate.

To evaluate the possibility that the observed changes in photosynthetic rate were caused by changes in alkalinity instead of salinity, photosynthesis measurements with and without the addition of HCO$_3^{-}$ were compared at five salinities ($0$, $10$, $20$, $30$, and $40\%$). The incubation period was 6 h, and irradiance was fixed at $85$ µmol photons m$^{-2}$ s$^{-1}$. The amount of HCO$_3^{-}$ added, ranged from $200$ to $1000$ M, was based on the $P_{\text{max}}$es from the salinity experiments, and oxygen was converted to carbon using a photosynthetic quotient of 1 (Littler and Arnold, 1985).

2.4. Statistical analysis

Two-way analysis of variance (ANOVA) was used to test if there were any significant effects of irradiance and salinity on growth of $G$. filicina (Sokal and Rohlf, 1980). A $t$-test was used to determine if the addition of HCO$_3^{-}$ induces differences in photosynthesis at each salinity tested.

3. Results

Although thalli of $G$. filicina grew at all salinity–irradiance combinations, significant differences in growth occurred (Fig. 1). The relationship between growth rate and salinity was best described by a hyperbolic function with the fastest growth found at $20\%$. Growth was reduced at other salinities, but, with the exception of freshwater, no morphological changes were observed. In freshwater, fronds started to bleach within 2 to 3 days and the plants died after 5 to 6 days (data not shown).

At irradiances below $100$ µmol m$^{-2}$ s$^{-1}$, growth increased with increasing irradiance. At the optimal irradiance of $100$ µmol m$^{-2}$ s$^{-1}$, growth averaged $13\%$ $d^{-1}$ for thalli incubated at $20\%$e salinity. When the irradiance was further increased to $135$ µmol m$^{-2}$ s$^{-1}$, somewhat less growth was observed (Fig. 1). The two-way ANOVA indicated
Fig. 1. Growth rate of *G. filicina* as a function of salinity under different irradiance levels. Various irradiance levels used are 5(●), 30(□), 55(△), 100(○) and 135(▲) μmol photons m$^{-2}$ s$^{-1}$, respectively. Error bar: ± 1 standard deviation.

Fig. 2. Photosynthesis vs. irradiance relationship in *G. filicina* under various salinities. The salinity settings are 0% (○), 10% (□), 20% (△), 30% (○), and 40% (△).
Table 1
The maximum photosynthetic capacity \( (P_{\text{max}}) \), respiration rate \( (R) \), \( R/P_{\text{max}} \) ratio, and half-saturation irradiance \( (I_s) \) of \textit{G. filicina} under various salinity treatments.

<table>
<thead>
<tr>
<th>Salinity (%)</th>
<th>( P_{\text{max}} ) (µmol O(_2) g fw(^{-1}) h(^{-1}))</th>
<th>( R ) (µmol O(_2) g fw(^{-1}) h(^{-1}))</th>
<th>( R/P_{\text{max}} ) ratio</th>
<th>( I_s ) (µmol photons m(^{-2}) s(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>15.3 (3.1)( ^{a} )</td>
<td>3.0 (2.7)</td>
<td>0.20</td>
<td>0.5(0.5)</td>
</tr>
<tr>
<td>10</td>
<td>89.0 (3.0)</td>
<td>9.6 (2.1)</td>
<td>0.11</td>
<td>7.9(1.4)</td>
</tr>
<tr>
<td>20</td>
<td>109.2 (5.4)</td>
<td>7.6 (2.7)</td>
<td>0.07</td>
<td>16.3(3.5)</td>
</tr>
<tr>
<td>30</td>
<td>55.1 (3.7)</td>
<td>6.7 (2.3)</td>
<td>0.12</td>
<td>10.6(3.5)</td>
</tr>
<tr>
<td>40</td>
<td>63.3 (6.1)</td>
<td>8.4 (2.4)</td>
<td>0.13</td>
<td>23.0(8.5)</td>
</tr>
</tbody>
</table>

\(^{a}\)Numbers in parentheses are the standard error (SE).

that the growth of thalli was significantly affected by both salinity \( (F = 50.71; df = 7.40; P < 0.001) \) and irradiance \( (F = 91.49; df = 4.40; P < 0.001) \). However, there was no significant interaction between the effects of salinity and irradiance on growth of thalli \( (F = 1.36; df = 28; P > 0.05) \).

Salinity also exerted strong influences on the maximum photosynthetic capacity \( (P_{\text{max}}) \) in \textit{G. filicina} (Fig. 2). All photosynthesis–irradiance curves showed a typical hyperbolic tangent shape with an initial increase followed by a saturated photosynthetic rate at high irradiance levels. The estimated \( P_{\text{max}} \) was low at 0%, 15.3 µmol O\(_2\) g fw\(^{-1}\) h\(^{-1}\), but reached a value of 109.2 µmol O\(_2\) g fw\(^{-1}\) h\(^{-1}\) at 20% (Table 1). When

![Fig. 3. \textit{G. filicina} photosynthetic rate with and without HCO\(_3\) enrichment at 5 different salinities. The enriched HCO\(_3\) concentration was 200, 1000, 1000, 700, and 750 µM for 0, 10, 20, 30, and 40%, respectively. Error bar represented ± 1 standard deviation. **: t-test \( P < 0.01 \).](image)
salinity was raised above 20%, $P_{\text{max}}$ started to decrease again. The estimated half-saturation irradiance ($I_{\text{c}}$) ranged between 0.5 and 23.0 μmol photons m$^{-2}$ s$^{-1}$ (Table 1).

At all salinity settings, the addition of HCO$_3^-$ did not stimulate net photosynthesis in the 6 h incubation period at any salinity tests (Fig. 3). An unexpected observation was that, at 20%, significantly lower rates occurred in HCO$_3^-$ treated samples.

Respiration of *G. filicina* was affected by salinity in a more complicated manner (Table 1). Thalli incubated at 0% had the lowest respiration rate at 3.0 μmol O$_2$ g fw$^{-1}$ d$^{-1}$. If this data point is temporarily ignored, the lowest respiration rate was observed at 30%, and the rate increased toward both high and low salinities.

4. Discussion

*G. filicina* exhibited broad salinity tolerances with respect to growth (Fig. 1). All thalli grew between 5% and 40% at all irradiance levels. The optimum growth was observed in the salinity range between 15% and 25%. The maximum growth rate was about 13% d$^{-1}$ at 20% and 100 μmol photons m$^{-2}$ s$^{-1}$. This maximum growth rate is much higher than the 4.5% d$^{-1}$ from *G. filicina* grown at 15% in Hawaii (Zablackis, 1987). In addition, Zablackis (1987) found no difference in growth rates at various salinities (15% to 35%). One explanation of the discrepancy in growth rate is that *G. filicina* raised in deeper outdoor tanks or tanks in a shaded place may receive much less irradiance than cultures raised in our walk-in incubator, and irradiance does have a strong influence on growth rate (Fig. 1). Another disagreement between the two studies is that Zablackis (1987) found salinity-dependent morphological differences, but the feature was not seen in our study even when the experiment was extended to 28 days. Perhaps *G. filicina* used in these two experiments were genetically different strains.

The effect of salinity on *G. filicina* photosynthesis was clearly seen from photosynthesis versus irradiance curves with widely different $P_{\text{max}}$ values (Fig. 2 and Table 1). This was not unexpected as several biochemical reactions in seaweed photosynthesis are sensitive to osmotic pressure. In *Enteromorpha*, *Porphyra* and *Ulva*, sensitivity occurs between plastoquinone and P700 (Kirst, 1989; Satoh et al., 1983), but it is unclear if salinity affects these two molecules in *G. filicina*. It is clear however that in *G. filicina*, the variation in $P_{\text{max}}$ is not a direct result of CO$_2$ limitation caused by decreased alkalinity in a lower salinity environment, as photosynthesis was not enhanced when HCO$_3^-$ was added to the incubation bottles (Fig. 3). Indeed, even when a higher HCO$_3^-$ concentration of 2.5 mM was evaluated in bottles containing 0, 20, and 40% seawater, we did not observe an elevated photosynthetic rate (data not shown).

The high values of $P_{\text{max}}$ occurred at 10 and 20% (Table 1). This range is somewhat lower than the 15 to 25% salinity range for optimal growth (Fig. 1). On the other hand, respiration rate of *G. filicina* fluctuated with salinity without an obvious trend (Table 1). This agrees well with the conclusion drawn by Koch and Lawrence (1987) who studied salinity effects on *Gracilaria verrucosa*. Lüning (1990) also concluded that respiration does not vary significantly with salinity for most seaweeds. In contrast, other examples indicate that respiration in seaweeds is highly sensitive to salinity (Kirst, 1995).
Although both $R$ and $P_{\text{max}}$ correlated rather poorly with the growth rate, the respiration to maximum photosynthetic capacity ratio ($R/P_{\text{max}}$ ratio) seems to have an inverse relationship with the growth of *G. filicina* (Fig. 1). The $R/P_{\text{max}}$ ratio has a minimum value of 0.07 at 20‰. It decreases with increasing salinity from 0‰ to 20‰, while starts to increase when salinity is higher than 20‰ (Table 1). Therefore, the $R/P_{\text{max}}$ ratio is a potentially useful indicator for the growth rate of *G. filicina*. Compared to the conventional method of determining growth rate using Eq. (1), the measurement of $R/P_{\text{max}}$ ratio is much faster and requires less plant material. However, further study is needed to evaluate the performance of $R/P_{\text{max}}$ ratio in other growth conditions such as nutrient limitation.

Compared to other seaweeds cultivated commercially, such as *Laminaria*, *Porphyra*, *Gracilaria*, and *Eucheuma* (Brinkhuis et al., 1984; Hanisak, 1987; Kain and Norton, 1990; Lüning, 1990; Lobban and Harrison, 1994), *G. filicina* has a lower requirement for light, and a somewhat lower salinity range for optimal growth. These traits make brackish water environments ideal sites for large scale cultivation of this species. The rope culture method, which is commonly employed in culturing *Laminaria* and *Undaria*, should also be suitable for *G. filicina* (Lobban and Harrison, 1994). As Iima et al. (1995) have improved the technique of regenerating new crusts, hatcheries can conveniently provide crust-covered ropes in large quantities. Tank culture is another method available to raise *G. filicina*. Usually, aeration is used to keep the thalli floating and to provide equal irradiance to all thalli. Although the costs of maintaining tank cultures are higher, our preliminary test indicated that the growth of *G. filicina* in tanks could be up to five times faster than that in a rope culture (Wong, unpublished result). In addition to salinity and irradiance, temperature and nutrients are also important to the growth of seaweeds. The effects of these factors on *G. filicina* needs to be evaluated in future in order to establish a successful cultivation system.

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**References**


