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Physiological differences in the growth of *Sargassum horneri* between the germling and adult stages

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Abstract The effects of temperature, irradiance, and daylength on *Sargassum horneri* growth were examined at the germling and adult stages to discern their physiological differences. Temperature–irradiance (10, 15, 20, 25, 30°C × 20, 40, 80 μmol photons $m^{-2}s^{-1}$) and daylength (8, 12, 16, 24 h) experiments were carried out. The germlings and blades of *S. horneri* grew over a wide range of temperatures (10–25°C), irradiances (20–80 μmol photons $m^{-2}s^{-1}$), and daylengths (8–24 h). At the optimal growth conditions, the relative growth rates (RGR) of the germlings were 21% day^{-1} (25°C, 20 μmol photons $m^{-2}s^{-1}$) and 13% day^{-1} (8 h daylength). In contrast, the RGRs of the blade weights were 4% day^{-1} (15°C, 20 μmol photons $m^{-2}s^{-1}$) and 5% day^{-1} (12 h daylength). Negative growth rates were found at 20 μmol photons $m^{-2}s^{-1}$ of 20°C and 25°C treatments after 12 days. This phenomenon coincides with the necrosis of *S. horneri* blades in field populations. In conclusion, we found physiological differences between *S. horneri* germlings and adults with respect to daylength and temperature optima. The growth of *S. horneri* germlings could be enhanced at 25°C, 20 μmol photons $m^{-2}s^{-1}$, and 8 h daylength for construction of *Sargassum* beds and restoration of barren areas.

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Introduction

The genus *Sargassum* (Fucales, Phaeophyceae) is distributed worldwide and forms dense monspecific or mixed stands in sublittoral zones (Yoshida 1983). Several species of *Sargassum* form underwater forests that provide habitat and spawning grounds to marine invertebrates and fish, thus playing an important role as a primary producer (Tsukidate 1992; Martin-Smith 1993; Choi et al. 2003).

Recently, *Sargassum* and *Ecklonia* beds have declined markedly, as a result of being covered by crustose red algae, and they have not recovered naturally in coastal areas of Korea. Such barren areas have been slowly expanding, and are known as "Isoyake" areas in Japan. Barren areas are created mainly by the intensive grazing pressures of herbivorous marine animals such as sea urchins and abalones (Harrold and Pears 1987; Largo and Ohno 1993). This increase in barren grounds in coastal areas has resulted in the decrease of marine resources such as fish, shellfish, and abalone. Thus, the construction of artificial underwater forests and the restoration of seaweed beds with *Eisenia*, *Ecklonia*, and *Sargassum* has been received particular attention from ecologists and phycologists (Yamauchi 1984; Largo and Ohno 1993; Terawaki et al. 2001; Choi et al. 2003).

Sargassum horneri (Turner) C. Agardh is a large dioecious alga growing up to 3–5 m long that forms underwater forest along the coasts of Korea (Kang 1968; Choi et al. 2003). Like other *Sargassum* spp., *S. horneri* is very important for marine ecosystems and is also used for fertilizer and food such as salads (Kang 1968). In coastal

areas of Korea, *S. horneri* grows rapidly from November to March, produces receptacles from late March to July, and necroses from late July to September. Thus, due to its fast growth rate, *S. horneri* could be used to construct underwater forests in seaweed barrens. For example, *S. horneri* seedlings grow up to 2–3 m long within 10 months after transplantation (Yamauchi 1984; Choi et al. 2003). To date, the life cycle and reproduction period of *S. horneri* have been examined by Japanese phycologists (Uchida 1993; Uchida and Arima 1993). In Korea, however, the effects of environmental factors on the growth of *S. horneri* germlings have not been examined, even though it is essential to know the optimal growth conditions for producing seedlings in order to construct *Sargassum* beds. Uchida and Arima (1993) observed two types of *S. horneri*: one produced embryos in the spring and the other in the autumn, and their growth responses to environmental factors were different. Thus, *S. horneri* growing on the rocky shores of Korea might have different physiological characteristics compared to Japanese *S. horneri*, and these algae may also differ in their germling and adult stages, since they grow during different seasons in the field. Therefore, the aim of this study was to examine the effects of environmental factors on the growth of *S. horneri* at the germling and adult stages, in order to determine the physiological differences between these stages.

Materials and methods

Germling experiments

Fertile female and male plants of *S. horneri* were taken from the intertidal zones of Huppo, Wuljin, in June 2005 for preliminary culture experiments. The present data were obtained from fertile plants of *S. horneri* collected at Jeonchonri, Wolsung, Kyungbuk, Korea (35° 47′ N, 129° 29′ E) in May 2006. To eliminate the effect of genetic difference on the growth of *S. horneri* germlings, one female and one male plant were used to release embryos, and their lengths were 175 cm and 135 cm, respectively. Fifty female receptacles with embryos, as well as male receptacles, were excised from parent plants and their lengths and widths were measured. They were then dried in an oven at 80°C for 5 days and weighed. The lengths, widths and dry weights of 50 air bladders of *S. horneri* were also measured by the same method.

For the germling culture, the female and male receptacles were cleaned several times with filtered seawater to remove diatoms and detritus attached to the receptacles. They were then put in a plastic tank to induce fertilization and release of embryos. After 24 h the receptacles were removed and a clean suspension of eggs was prepared as described by Creed et al. (1996).

A zygote suspension was inoculated into 20 Petri dishes, each containing eight glass slides cut to 2.5×2.0 cm and 30 mL autoclaved seawater. After 24 h, we selected the 114 slides on which the germlings were most evenly distributed to use in the daylength and temperature–irradiance experiments. The settlement density of the germlings was 16.6± 5.74 germlings cm^{-2} (mean±SD, n=30).

Two experiments were carried out in the laboratory to investigate the influence of environmental factors on the growth of *S. horneri*. In the temperature–irradiance experiment, a total of 45 beakers were used and each beaker contained 150 mL PESI medium (Tatewaki 1966) to which GeO_2 (2 mL) per 1 L PESI had been added to inhibit the growth of diatoms and protozoa. Two slides with germlings were put into each beaker, and the beakers were then placed within five incubators that had been set up at 10, 15, 20, 25, and 30°C and 20, 40, and 80 μmol photons $m^{-2}s^{-1}$ with a 16:8 h light:dark cycle, and cultured for 12 days. Light was provided by 40 W cool-white fluorescent tubes and the level of irradiance was measured using a digital illumination meter (DX-200). Different irradiance levels (20, 40, and 80 μmol photons $m^{-2}s^{-1}$) were created by covering the beakers with black plastic mesh. This experiment was repeated three times and the culture medium was changed every 4 days during the 12-day culture period.

After 12 days, the lengths of 30 germlings, excluding the rhizoids, were measured in each replicate beaker. The relative growth rate (RGR % day^{-1}) was calculated with the mean length for each replicate using the following equation (Rueness and Tananger 1984):

$$RGR\left(\%\, day^{-1}\right) = 100 \ln\left(L_t/L_o\right)/t$$

where L_o is the initial plant length, L_t is the final length after t days, and t is the number of days.

For the daylength experiment, 12 beakers, each containing two cut slides with attached germlings and 150 mL PESI medium with GeO_2 (2 mL), were prepared as described above. The beakers were placed in four incubators that had been set up at 8, 12, 16, and 24 h light per 24 h. The plants were cultured for 12 days at 20±1°C and 40 μmol photons $m^{-2}s^{-1}$. The experiments were replicated three times and the culture medium changed every 4 days throughout the experimental period.

Adult stage experiments

Twenty vegetative plants of *S. horneri* were collected from the intertidal zone of Jeonchonri, Wolsung, Kyungbuk, Korea in October 2006. The plants were transported to the laboratory and their lengths and wet weights were measured.

The vegetative plants ranged between 23 and 59 cm in length and 2.84–23.34 g in wet weight. The average length and wet weight of the vegetative plants were 35.90±9.95 cm and 9.96±6.17 g (mean±SD, n=20 individuals), respectively. The apical blades from one vegetative plant were cut and rinsed several times with filtered seawater to remove diatoms and detritus attached to the blades. The healthy apical blades, 10 mm in length and 5.66±1.51 mg (mean±SD, n=30 individuals) in wet weight, were excised and kept for 2 days to reduce any negative effects of the cutting.

We used a total of 36 beakers, each of which contained 150 mL PESI medium to which GeO_2 (2 mL per 1 L PESI) had been added to inhibit the growth of diatoms and protozoa. Three apical blades were put into each beaker and placed in four incubators that had been set up at 10, 15, 20, and 25°C and 20, 40, and 80 μmol photons $m^{-2}s^{-1}$ and 16:8 h LD (light:dark), and cultured for 12 days. The experiments were replicated three times and the culture medium was changed every 4 days throughout the experimental period.

To examine the effects of daylength on blade growth we prepared 12 beakers that contained three apical blades (10 mm long) and 150 mL PESI medium with GeO_2. Three apical blades were put into each beaker and placed in four incubators that had been set up at 8, 12, 16, and 24 h light/24 h. The plants were cultured for 12 days at 40 μmol photons $m^{-2}s^{-1}$ and 20±1°C. The RGR (% day^{-1}) was calculated using the mean blade length and weight of each replicate with the equation described above.

Statistical analyses

Statistical analyses were carried out using STATISTICA version 5.0 software. A two-way ANOVA was used to test the effects of temperature and irradiance on the RGR of germling lengths, blade lengths, and blade weights. A one-way ANOVA was used to determine the differences in RGR for germling lengths, blade lengths, and blade weights of specimens cultured at four different daylengths. A Tukey test was applied when significant differences were detected between the means (Sokal and Rohlf 1995). Homogeneity of the variance was tested using Cochrane's test (Underwood 1997).

Results

Characteristics of parent plants

The female receptacles of *S. horneri* were cylindrical but much thicker and shorter than the male receptacles. The lengths of the female receptacles ranged between 13 and 24 mm and the average length was 19.46±1.80 mm (mean±SD, n=50 receptacles). The widths of the female receptacles with attached germlings were 3–5 mm and the average width was 3.70±0.51 mm (n=50 receptacles). After germling release, the receptacle width was about 2 mm. The dry weight of each receptacle was 10.47±7.82 mg (mean±SD, n=50). The male receptacle was 31.16±13.40 mm (n=50) in length and was 2.77±0.72 mm (n=50) in width.

The air bladder of *S. horneri* was also cylindrical with a length of 10.48±1.63 mm (mean±SD, n=50 individuals), and a width of 1.26±0.31 mm (n=50). The mean dry weight of the air bladders with a serrated leaf attached to the top was 1.90±0.86 mg (mean±SD, n=50). The mean length of the serrated leaves of the air bladders was 12.94±3.11 mm (mean±SD, n=50).

Effects of temperature and irradiance on growth

Germling stages

The germlings of *S. horneri* grew at temperatures ranging from 10 to 25°C and irradiances between 20 and 80 μmol photons $m^{-2}s^{-1}$ after 12 days (Fig. 1). Germling growth was inhibited between 25–30°C and, at 30°C, approximately 85% of the germlings died when no rhizoids were produced within 12 days, irrespective of the irradiance levels. Thus, all data obtained from 30°C were excluded from the statistical analyses. The germling lengths ranged between 0.5 and 2.93 mm with a maximal length of 2.93±0.14 mm (mean±SE, n=3 replicates) at 25°C and 20 μmol photons $m^{-2}s^{-1}$. The relative growth rates of *S. horneri* were between 6.22 and 21.17 % day^{-1}, with a maximal rate of 21.17±0.38% day^{-1} at 25°C and 20 μmol photons $m^{-2}s^{-1}$.

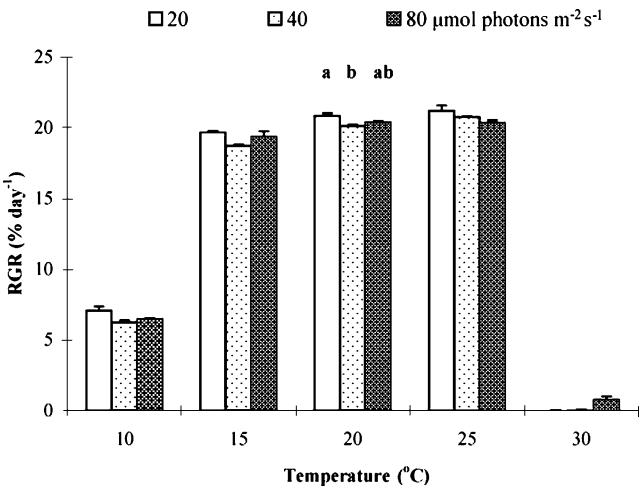

Fig. 1 Mean(±SE) relative growth rate (RGR; % day^{-1}) of *Sargassum horneri* germlings. Seaweeds were cultured at various temperatures and irradiances for 12 days. Each mean value is based on three replicates. Lower case letters indicate significant differences based on a Tukey test

The RGRs of the *Sargassum* germlings were greater at higher temperatures (15–25°C) than at 10°C, and were found to be significantly different among the various temperatures ($P<0.001$). A Tukey test revealed that the RGRs were significantly different between temperatures, except between 20 and 25°C (Fig. 1). The germlings of *S. horneri* grew faster at 20 μmol photons $m^{-2}s^{-1}$ than at 40 and 80 μmol photons $m^{-2}s^{-1}$ ($P<0.001$), but no differences were found at the higher irradiances ($P>0.05$). There was no significant interaction between the effects of temperature and irradiance levels on the RGR of the *Sargassum* germlings ($P=0.34$).

Adult stages

After 12 days, the RGRs of *S. horneri* were very low, ranging from 0.08 to 1.88 % day^{-1} for blade length and between 0.11 and 4.46% day^{-1} for blade weight (Fig. 2a,b). For blade length, we found significant differences in the RGRs of *S. horneri* between irradiance levels, but not for temperature (Table 1). The RGR of *S. horneri* for blade length was greater at low irradiances (20, 40 μmol photons $m^{-2}s^{-1}$) than at 80 μmol photons $m^{-2}s^{-1}$ (Table 1). For *S. horneri* blade length, a negative growth rate was found at 25°C and 80 μmol photons $m^{-2}s^{-1}$. This indicates that the growth of *S. horneri* blades is inhibited at high irradiance levels and high seawater temperatures. The RGR for blade length was significantly greater at 40 than at 80 μmol photons $m^{-2}s^{-1}$. For blade weight, the RGRs were greater than those of blade length. This result indicates that *Sargassum* blades grew mainly in width and thickness at this life stage. Also, there were significant differences in the relative growth rates of blade weight between temperatures and irradiance levels (Table 1). The growth in blade weight was superior at lower temperatures (10, 15°C) than at higher temperatures (20, 25°C). The RGR for blade weight was significantly greater at 15°C, the optimal temperature, than at 20 and 25°C (Tukey test). With respect to the adults, negative RGR values were observed at an irradiance of 80 μmol photons $m^{-2}s^{-1}$ and temperatures of 20 and 25°C after 12 days. In the temperature-irradiance experiment, maximal growth occurred at 15°C and 20 μmol photons $m^{-2}s^{-1}$ for both blade length and weight.

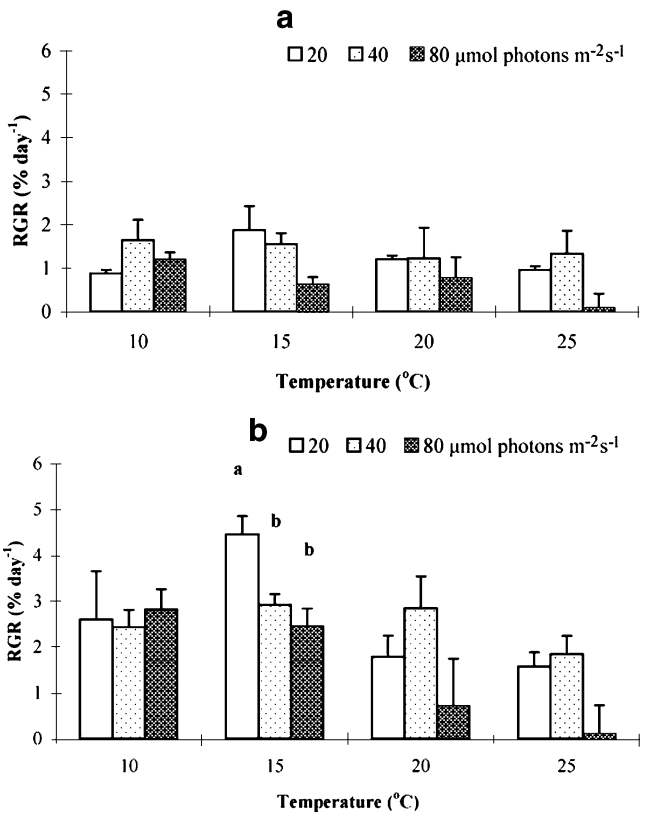

Fig. 2 Mean (±SE) RGR (% day^{-1}) of *S. horneri* for blade length (**a**), and blade weight (**b**). Seaweeds were cultured at various temperatures and irradiances for 12 days. Each mean value is based on three replicates. Lower case letters indicate significant differences based on a Tukey test

Effects of daylength on growth

Germling stages

The initial length of the *S. horneri* germlings was 230.55± 41.88 μm (mean±SD, $n=80$ spores). The germlings grew well in a wide range of daylengths ranging between 8 and 24 h and grew to lengths of 1.02–1.28 mm after 12 days. The RGRs for germling length were from 11.53 to 13.42% day^{-1} (Fig. 3), and the RGRs of germling length were significantly different among daylengths ($F_{3,8}=29.51$, $P<0.001$). The optimal daylength for germling growth was 8 h, and the growth of the germlings was slowed at 24 h of daylength. A Tukey test showed that the RGRs of the germlings were significantly greater at 8 h than at 24 h of daylength (Fig. 3).

Adult stages

After 12 days the RGRs of the *S. horneri* blades were between 0.35 and 2.72 % day^{-1} for length, and 2.25 and 4.93% day^{-1} for weight in the range of daylengths between 8 h and 24 h (Fig. 4a,b). The maximal RGR was observed at 12 h of daylength for both blade length and weight. A Tukey test revealed that the blade length RGR decreased significantly only at 24 h of daylength ($F_{3,8}=7.18$, $P<0.05$), but no differences were found for blade weight among the daylengths ($F_{3,8}=6.48$, $P<0.05$). After 16 days the blades of *S. horneri* began to necrose from the apical area, most severely at 24 h of daylength as compared to the blades of other daylengths.

Table 1 Results of two-way ANOVA and Tukey tests for the effects of temperature and irradiance on relative growth rate (RGR, % day^{-1}) of *Sargassum horneri* blades grown at various temperature and irradiance levels

Factor	df	MS	F	*P*	MS	F	*P*
		Length			Weight		
Temperature	3	0.53	1.20	0.33	7.67	7.26	<0.01
Irradiance	2	1.91	4.25	<0.05	4.33	4.09	<0.05
Interaction	6	0.42	0.93	0.49	1.71	1.62	0.18
Residuals	24	0.45			1.06		
Tukey test (P=0.05)							
Temperature		10=15=20=25			10=15=20>25, 15>20>25, 20=25		
Irradiance		20=40=80, 40>80			20=40>80, 40=80		

Discussion

The growth of *Sargassum horneri* was affected by daylength at both the germling and adult stages. The germlings of *S. horneri* grew better under 8 h of daylength than under constant light. This growth trend of the *S. horneri* germlings was similar to shoot formation of *S. horneri* (Uchida 1993), and to the main branch elongation and receptacle growth of *S. muticum* (Hales and Fletcher 1990; Uchida et al. 1991). Also, the length and weight growths of *S. horneri* blades were maximal at 12 h of daylength (neutral day). These results indicate that the effects of daylength on the growth of *S. horneri* are slightly different between the germling and adult stages. Also, these growth responses to daylength are very important for understanding the growth patterns of natural *S. horneri* populations. In Korea, daylength ranges between 9.5 and 14.5, being maximal in June and minimal in December (Choi 1996). In field populations of *S. horneri*, the main growth period for the juveniles and adults is from October to March, with 9.5–11.5 h daylength. Thus, the results found with culture experiments are very similar to the daylength growth responses of germlings and adults in natural populations.

Temperature and irradiance are very important environmental factors influencing the growth of germlings and adults in *Sargassum* spp. (Hales and Fletcher 1989, 1990). In the present study, the maximum growth of *S. horneri* germlings was 20.74% day^{-1}(RGR) at 25°C, just as in *S. muticum* (Hales and Fletcher 1989). On the other hand, the average RGRs of *S. horneri* for blade length and blade

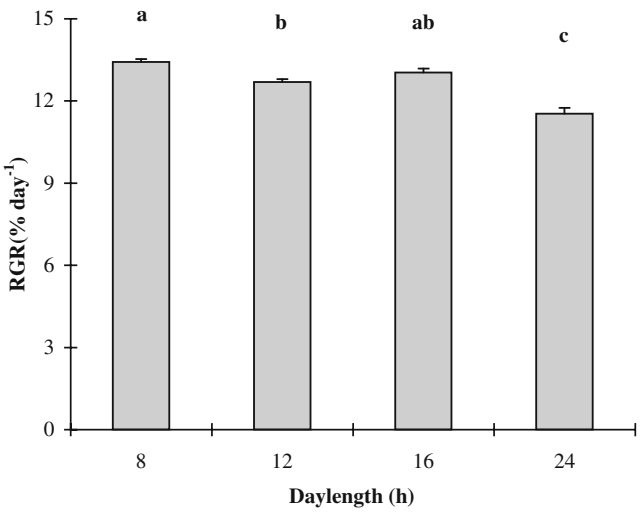

Fig. 3 Effects of daylength on the relative growth rate of *S. horneri* germlings. Seaweeds were cultured at various irradiance levels for 12 days. *Bars* show standard errors (n=3 replicates). Lower case letters indicate significant differences based on a Tukey test

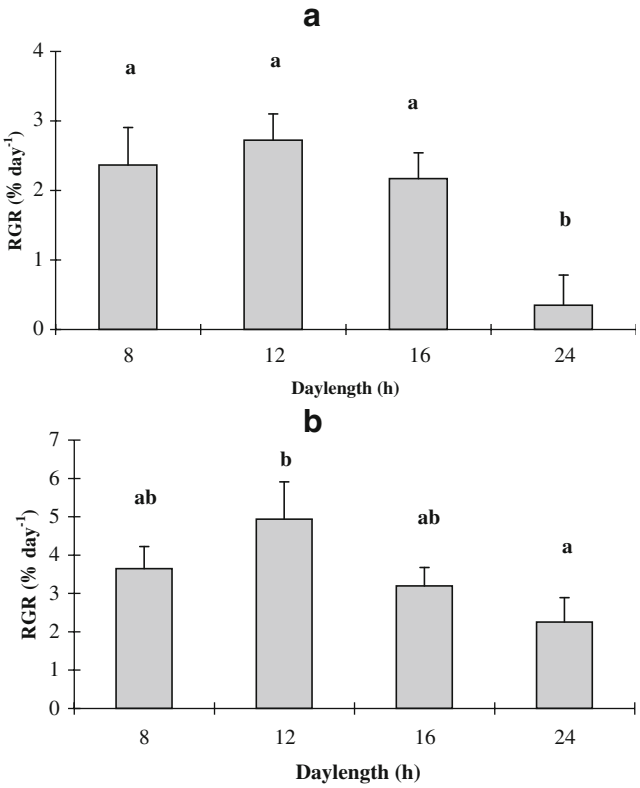

Fig. 4 Effects of daylength on the relative growth rate of *S. horneri* for blade length (**a**), and blade weight (**b**). Seaweeds were cultured at various irradiance levels for 12 days. *Bars* show standard errors (n=3 replicates). Lower case letters indicate significant differences based on a Tukey test

weight were very low, and the maximal value for blade weight was 4.46% day^{-1}(RGR) at 15°C. Hales and Fletcher (1989, 1990) found that maximal growth of *S. muticum* germlings and adults occurred at different salinity and irradiance conditions, but their optimal temperature for growth was exactly the same. Such different temperature requirements for optimal growth at germling and adult stages of *S. horneri* may closely relate to seawater temperature. The average seawater temperature of the sampling area ranged from 7.62°C in February to 23.94°C in September 2006. Thus, the fronds of *S. horneri* could grow well at 15°C, which corresponds to the seawater temperature of the sampling area in November. Germling growth was significantly greater at 20 μmol photons $m^{-2}s^{-1}$ than at 40 and 80 μmol photons $m^{-2}s^{-1}$, indicating that *S. horneri* germlings require a very low irradiance for growth. This coincides with the light conditions of sublittoral zones, where *S. horneri* germlings grow in the field.

In the present study we found remarkable physiological differences in the growth of *S. horneri* between the germling and adult stages. At optimal growth conditions the RGRs of the germlings were 21% day^{-1} (25°C, 20 μmol photons $m^{-2}s^{-1}$) and 13% day^{-1} (8 h daylength), whereas those of the blade weights were 4% day^{-1} (15°C, 20 μmol photons $m^{-2}s^{-1}$) and 5% day^{-1} (12 h daylength), indicating that the RGRs of the germlings were 3–5 times greater than those of the blades. Similar growth rates were also found in transplanted *S. horneri* individuals as 3.3% day^{-1} for length and 4.7% day^{-1} for frond weight (Yamauchi 1984). *Sargassum* germlings grew faster than adult plants. For instance, the RGRs of transplanted *S. muticum* juveniles were 2.4% in length and 0.3% in weight, but its germlings grew 28% day^{-1}(Yamauchi 1984; Hales and Fletcher 1989).

Sargassum germlings grew faster than adults but this does not mean that germlings will be more successful for construction of *Sargassum* beds because germlings are vulnerable to invertebrate grazers (Choi et al. 2003). Transplantation of live adults to barren areas might be a better method to establish seaweed beds despite their lower growth rates, because they are more resistant to grazing, provide immediate habitat to marine animals, and act as a seed bank producing embryos. However, transplanting live adults is very difficult because of cost and labor requirements. Largo and Ohno (1993) described various construction methods of artificial seaweed beds. They are divided into spore techniques (spore dispersal, spore-bag, and rope-seeding method) and vegetative plant transplanting techniques (concrete block, threading, and gravel-bag methods). To date, the spore-bag and rope-seeding methods have been used in order to create *Sargassum* beds (Largo and Ohno 1993; Choi et al. 2003). Choi et al. (2003) found that *S. horneri* juveniles transplanted on tiles were completely consumed by herbivores (sea urchins and snails), but juveniles transplanted on iron pipes grew up to 313 cm in length and formed underwater forests. Thus, the construction of *S. horneri* beds by transplantation of juveniles is possible under lower grazer density as on iron pipes (Choi et al. 2003). In conclusion, we found physiological differences between *S. horneri* germlings and adults with respect to daylength and temperature optima. The growth of *S. horneri* germlings for construction of *Sargassum* beds and restoration of barren areas could be enhanced by the application of the following conditions: 25°C, 20 μmol photons $m^{-2}s^{-1}$ and 8 h daylength.

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