

## Physiological differences in the growth and maturation of *Eisenia bicyclis* and *Ecklonia cava* gametophytes in Korea

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**Abstract** The objective of this study was to examine the effects of temperature and light intensity on growth of female gametophytes of *Eisenia bicyclis* and *Ecklonia cava* and responses of these female gametophytes to Fe addition and daylength. Female gametophytes of each species were cultured at four temperatures (10, 15, 20, and 25°C) and under a combination of four light intensities (10, 20, 40, and 80  $\mu\text{mol photons}/(\text{m}^2\cdot\text{s})$ ) with two temperatures (15 and 20°C for *Ei. bicyclis*; 20 and 25°C for *Ec. cava*) to clarify their optimal growth conditions. Growth and maturation of female gametophytes of these two species under a combination of five Fe-EDTA concentrations (0, 1, 2, 4, and 8  $\mu\text{mol/L}$ ) and three daylengths (10, 12, and 14 h) were also examined. The growth of *Ei. bicyclis* gametophyte was maximal at approximately 15–20°C, 20  $\mu\text{mol photons}/(\text{m}^2\cdot\text{s})$ , Fe-EDTA concentration of 8  $\mu\text{mol/L}$  and daylengths of 12–14 h. While *Ec. cava* gametophytes showed optimal growth at approximately 20–25°C, 20  $\mu\text{mol photons}/(\text{m}^2\cdot\text{s})$ , Fe-EDTA concentration of 8  $\mu\text{mol/L}$  and daylength of 14 h. Maturation of gametophytes was enhanced at Fe-EDTA concentration of 4  $\mu\text{mol/L}$  for *Ei. bicyclis* and at 2  $\mu\text{mol/L}$  for *Ec. cava*. In conclusion, optimal growth temperatures and Fe-EDTA concentrations for maturation of *Ei. bicyclis* and *Ec. cava* gametophytes were different. Higher optimal growth temperature for *Ec. cava* gametophytes may contribute to its wider geographical distribution compared to *Ei. bicyclis* which has restricted habitats in Korea. This suggests that addition of Fe ion could be used to recover beds of these two species in barren grounds of Korea.

**Keyword:** *Ecklonia cava*; *Eisenia bicyclis*; gametophyte; growth; maturation

### 1 INTRODUCTION

*Eisenia bicyclis* (Kjellman) Setchell and *Ecklonia cava* Kjellman are two large brown algae belonging to Lessoniaceae of Laminariales. They are perennial species with about 3–7 years of lifespan (Yoshida, 1970; Kawashima, 1993). These two species have a typical heteromorphic life cycle with macroscopic sporophytes and microscopic gametophytes (Hori, 1993). Sporophytes of these two species can grow up to more than 1 m in length (Kang et al., 2001) and form dense populations called “marine forest” on the coast of Korea. Marine forests are ecologically important roles as nursery and breeding grounds for various marine animals. They act as important primary producers in coastal ecosystems (Levitt, 1993; Eriksson et al., 2006; Lorentsen et al., 2010). However, beds of *Ei. bicyclis* and *Ec. cava* have declined remarkably in recent years. Major causes of

such decline are thought to be temperature increase, nutrient deficiency, and grazing pressure (Nakayama and Arai, 1999; Terawaki et al., 2001; Muraoka, 2008; Arakawa et al., 2013; Gao et al., 2016).

Geographical distribution is very different between *Ei. bicyclis* and *Ec. cava* in Korean waters. *Ei. bicyclis* is restricted along the coasts of Ulleungdo and Dokdo Islands while *Ec. cava* has wide distribution, ranging from the coast of Jeju Island of the South Sea to Ulleungdo and Dokdo Islands (Kim and Kim, 2000; Choi et al., 2009). Based on biomass and population density on the coasts of Ulleungdo and Dokdo Islands, Korea, *Ei. bicyclis* is dominant within depth ranging from 2 to 10 m (optimal depth 5 m) while *Ec. cava* is dominant with depth ranging from 2 to 25 m having optimal depth 10–25 m (Kang et al., 2001; Choi et al.,

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2009). Such differences in the geographical and vertical distribution of these two species have been thought to be due to the different physiological responses of gametophytes or sporophytes to seawater temperature and light condition (Maegawa et al., 1988; Morita et al., 2003a; Rothäusler et al., 2011). Young sporophytes of *Ec. cava* could survive better at a high temperature of 30°C than those of *Ei. bicyclis* (Arakawa et al., 2013). However, comparison of their physiological responses to a temperature at the gametophyte stage that shows greater sensitivity to environmental conditions has not been reported yet.

In the life cycle of kelp species, growth and maturation of gametophytes are crucial for producing natural populations (Altamirano et al., 2003; Wiencke et al., 2007). Previous studies have elucidated temperature and light requirements for the growth and maturation of *Ec. cava* gametophytes for commercial cultivation and seedling production (Wi et al., 2008; Hwang et al., 2010). Germination and growth of *Ec. cava* gametophytes are inhibited by increasing  $p\text{CO}_2$  of seawater (Oh et al., 2015). However, up to date, very few studies have focused on the influence of environmental factors such as temperature, light, and nutrient on the growth and maturation of *Ei. bicyclis* gametophytes, especially in Korea. Therefore, more information on the basic physiology of *Ei. bicyclis* is strongly desired to clarify the cause of its population reduction and the potential difference compared to *Ec. cava* in Korean waters.

A rapid population growth of *Saccharina japonica* has been confirmed by adding dissolved iron concentrations at a barren ground with lower iron concentration (<2 nmol/L) in Japan (Matsunaga et al., 1994). Similarly, the growth of germlings and juveniles of four *Sargassum* species can be stimulated by providing dissolved Fe species (Nagai et al., 2014; Miki et al., 2016). Iwai et al. (2015) have reported that resistance of seaweeds to grazing can be significantly decreased under lower dissolved iron concentration. In addition, oogonia production of *Saccharina angustata* (= *Laminaria angustata*) and *S. japonica* can be significantly increased from 0.4%–0.9% (no addition of Fe ion) to 98.9%–99.4% by treatment of adding Fe chelate (Motomura and Sakai, 1981, 1984). Therefore, levels of dissolved iron in coastal seawater could be one of the most important factors in maintaining and restoring kelp beds in Korean coastal areas.

In the present study, we examined the effects of seawater temperature, light condition (light intensity and day length), and micronutrient (Fe-EDTA) on the

growth and maturation of female gametophytes of *Ei. bicyclis* and *Ec. cava* to determine physiological differences between these two species for a possible interpretation of their geographical and vertical distributions in Korea. We tested the following questions: (1) do temperature, light intensity, day length, and Fe-EDTA concentration have a significant effect on the growth of female gametophytes of these two species? (2) do female gametophytes of these two species have different requirements for temperature, light intensity, and Fe ion concentration for maturation?

## 2 MATERIAL AND METHOD

### 2.1 Sample collection and production

Fertile *Ei. bicyclis* sporophytes were collected from natural populations in Dokdo, Ulleungdo, Gyubuk (37°23'N, 131°86'E) in January 2016. Fertile *Ec. cava* sporophytes were collected from natural populations in Jangsan, Shinan, Cheonam (34°37'N, 126°46'E) in October 2015. Samples were transported quickly to the laboratory in cool boxes filled with seawater. Mature sorus parts were cut and rinsed several times with autoclaved seawater to remove diatoms and detritus. After removing moisture using paper towel and drying for 2 h, these sorus parts were placed in beakers containing 100 mL of autoclaved seawater and incubated at temperature of 10°C, light intensity of 20  $\mu\text{mol photons}/(\text{m}^2 \cdot \text{s})$ , and a light:dark cycle of 10 h:14 h for 24 h to release zoospores. Zoospore suspension (5 mL) was then inoculated into Petri dishes (15 cm in diameter) containing cut slide glass (2.5 cm×2.5 cm) and PESI medium (30 mL). Gametophytes attached to slides (20–30 inds./ $\text{cm}^2$ ) were stock-cultured at a temperature of 20°C and light intensity of 20  $\mu\text{mol photons}/(\text{m}^2 \cdot \text{s})$  with a light:dark cycle of 12 h:12 h for 15 days for the following experiments.

### 2.2 Effects of temperature and light intensity on growth

To examine the effect of temperature on gametophyte growth of these two species, stock-cultured gametophytes of these two species were cut using High-functional Tissue Grinders (1 mL, WHEATON, USA) in autoclaved seawater. Gametophyte suspension (10 mL) was inoculated using a pipette into Petri dishes (15 cm in diameter) containing slides and autoclaved seawater. On the

next day, a culture experiment at four temperatures (10, 15, 20, and 25°C) was performed for 10 days. A total of twelve Petri dishes (5 cm in diameter) were used for each species, with three replicates for each temperature. Each Petri dish contained one slide with gametophytes and PESI medium (10 mL). During the experimental period, the light intensity of 20  $\mu\text{mol photons}/(\text{m}^2\cdot\text{s})$  and light:dark cycle of 12 h:12 h were held constant. The culture medium was renewed every two days. After 10 days, photography for gametophytes was made using a digital camera attached to a light microscope. Compared to male gametophytes having many branches and irregular growth patterns, female gametophytes have simple morphological structure and growth pattern. Therefore, female gametophytes were selected as a target to monitor their growth. After randomly selecting 30 female gametophytes, their lengths were measured using Image J program. The relative growth rate (RGR) was calculated using the average length of 30 female gametophytes for each temperature treatment with the following equation (Serisawa et al., 2002):

$$\text{RGR (\%/d)} = 100 (\ln L_2 - \ln L_1) / T_2 - T_1,$$

where  $L_2$  was the final length of female gametophytes after the experiment,  $L_1$  was the initial length of female gametophytes, and  $T_2 - T_1$  was the number of days.

To examine the combined effects of temperature and light intensity on gametophyte growth of these two species, they were incubated for 10 days at two temperatures (15 and 20°C for *Ei. bicyclis*; 20 and 25°C for *Ec. cava*) and four light intensities (10, 20, 40 and 80  $\mu\text{mol photons}/(\text{m}^2\cdot\text{s})$ ). A total of 24 Petri dishes (5 cm in diameter) including three replicates for each treatment were prepared for each species. Each Petri dish contained one slide with gametophytes and PESI medium (10 mL). Photoperiod was set as 12 h:12 h L:D. The culture medium was renewed every two days. After 10 days, lengths of 30 female gametophytes was measured and RGR were calculated in the same way as described above.

### 2.3 Effects of Fe-EDTA concentration and daylength on growth and maturation

To examine the effects of Fe-EDTA concentration and daylength on growth and maturation of gametophytes of these two species, they were incubated for 10 days at five Fe-EDTA concentrations (0, 1, 2, 4, and 8  $\mu\text{mol/L}$ ) and three daylengths (10,

12, and 14 h). A total of 45 Petri dishes (5 cm in diameter) were used for each species, with three replicates for each treatment. Each Petri dish contained one slide with gametophytes and 10 mL of medium with different Fe-EDTA concentrations. Different Fe-EDTA concentrations were made by dilution method using a stock solution (1 mmol/L). The stock solution was made by dissolving 0.039 g of  $\text{Fe}(\text{SO}_4)_2\text{NH}_4\cdot 6\text{H}_2\text{O}$  (Sigma Co., USA) and 0.029 g of EDTA in 100 mL of low nutrient seawater (natural Atlantic seawater). The low nutrient seawater had nutrients less than one micromole per liter. It was used as a blank or for the preparation of standard solutions. It was made by OSIL Company (<http://www.osil.co.uk>) of the United Kingdom. In this experiment, optimal temperature and light intensity for the growth of female gametophytes of *Ei. bicyclis* (20°C and 20  $\mu\text{mol photons}/(\text{m}^2\cdot\text{s})$ ) and *Ec. cava* (25°C and 20  $\mu\text{mol photons}/(\text{m}^2\cdot\text{s})$ ) were used. Culture medium change and growth estimation were the same as described earlier. The maturity of female gametophytes was also determined after 10 days using a light microscope. Maturity was reported as a percentage of female gametophytes that had released an egg or formed a sporophyte.

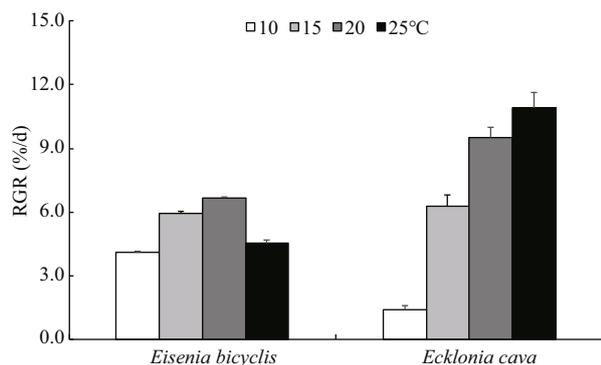
### 2.4 Statistical analysis

Two-way analysis of variance (ANOVA) was used to compare RGRs among female gametophytes of these two kelp species under different temperatures, test the effects of temperature and light intensity on RGR of female gametophytes, and effects of Fe-EDTA concentration and daylength on RGR and maturity of female gametophytes of these two kelps. A Turkey HSD test was applied when significant differences were detected between the means (Sokal and Rohlf, 1995). Prior to analysis, homogeneity of variance was determined using Cochran's test. All statistical analyses were performed using Statistica version 7.0 software.

## 3 RESULT

### 3.1 Growth comparison of these two kelps under different temperatures

Female gametophytes of *Ec. cava* showed significantly greater growth than those of *Ei. bicyclis* within 10 days ( $F_{1,16}=27.09$ ,  $P<0.001$ ; Fig.1). RGRs of female gametophytes of these two species were significantly different between temperature treatments



**Fig.1** Relative growth rate (RGR, %/d) of female gametophytes of *Eisenia bicyclis* and *Ecklonia cava* cultured for 10 days under four temperatures

Data represents mean±SE (n=3 replicates).

**Table 1** Analysis of variance (two-way ANOVA) for the effects of temperature and light intensity on the growth of female gametophytes of *Eisenia bicyclis* and *Ecklonia cava*

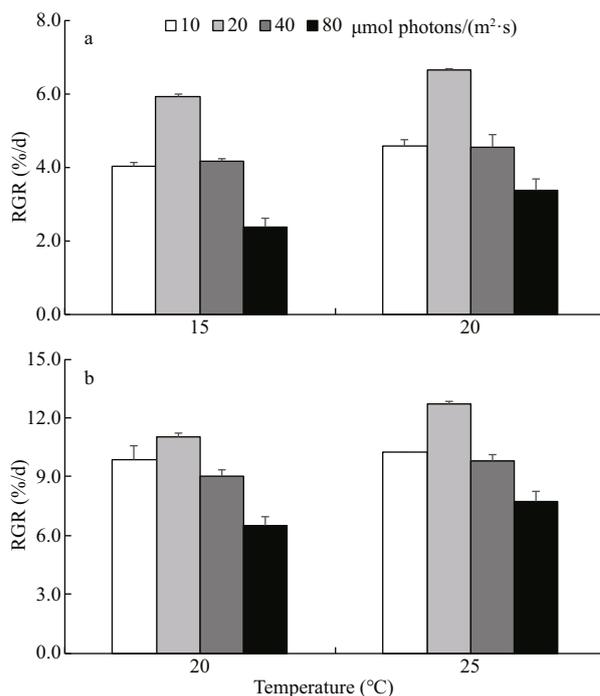
Factors	df	<i>Eisenia bicyclis</i>			<i>Ecklonia cava</i>		
		MS	F	P	MS	F	P
Temperature (°C)	1	2.75	5.48	<0.05	6.13	13.88	<0.001
Light intensity	3	10.82	21.57	<0.001	23.04	52.19	<0.001
Interaction	3	0.02	0.05	0.99	0.49	1.10	0.38
Error	16	0.50			0.44		
Tukey test (P=0.05)							
Temperature (°C)		15<20			20<25		
Irradiance		10=40=80<20			80<10=40<20		

( $F_{3,16}=52.17$ ,  $P<0.001$ ). Significant interaction between temperature and species was also observed ( $F_{3,16}=32.54$ ,  $P<0.001$ ).

After 10 days, RGRs of *Ei. bicyclis* gametophytes ranged from 3.91%–6.48%/d at experimental temperatures tested. These values at 15 and 20°C were significantly higher than those at 10 and 25°C (Fig.1). RGRs of *Ec. cava* gametophytes were in the range of 1.23%–10.87%/d. These values at 20 and 25°C were significantly higher than those at 10 and 15°C.

### 3.2 Effects of temperature and light intensity on growth

Female gametophytes of *Ei. bicyclis* at 20°C had significantly higher RGRs than those at 15°C while those of *Ec. cava* at 25°C had significantly higher RGRs than those at 20°C (Table 1). Light intensity also significantly affected the growth of female gametophytes for each species. However, a significant interaction between temperature and light intensity



**Fig.2** Relative growth rate (RGR, %/d) of female gametophytes of *Eisenia bicyclis* (a) and *Ecklonia cava* (b) cultured for 10 days at four light intensities and two temperatures

Data represents mean±SE (n=3 replicates).

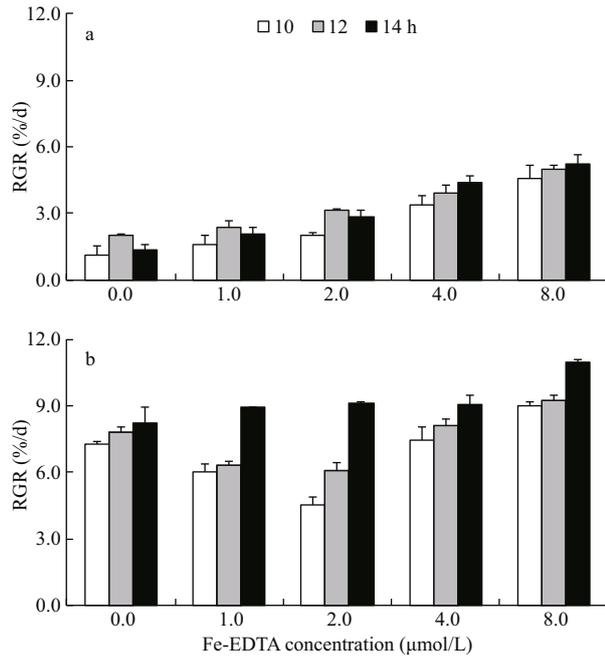
was not observed. For both species, RGRs of female gametophytes at 20 μmol photons/(m²·s) were significantly higher than those at other three light intensities (Fig.2a, b).

### 3.3 Effects of Fe-EDTA concentration and daylength on growth

Addition of Fe-EDTA and increased daylength significantly stimulated the growth of female gametophytes of both *Ei. bicyclis* and *Ec. cava* (Fig.3a, b; Table 2). However, a significant interaction between Fe-EDTA concentration and daylength was only observed for *Ec. cava*. For both species, RGRs of female gametophytes at Fe-EDTA concentration of 8 μmol/L were significantly higher than those at other four Fe-EDTA concentrations. Female gametophytes of *Ei. bicyclis* at daylength of 12 h or 14 h had significantly higher RGRs than those at daylength of 10 h while those of *Ec. cava* at daylength of 14 h had significantly higher RGRs than those at daylength of 12 h or 10 h.

### 3.4 Effects of Fe-EDTA concentration and daylength on maturation

The maturity of female gametophytes of *Ei. bicyclis*



**Fig.3** Relative growth rate (RGR, %/d) of female gametophytes of *Eisenia bicyclis* (a) and *Ecklonia cava* (b) cultured for 10 days at five Fe-EDTA concentrations and three daylengths  
Data represents mean±SE (n=3 replicates).

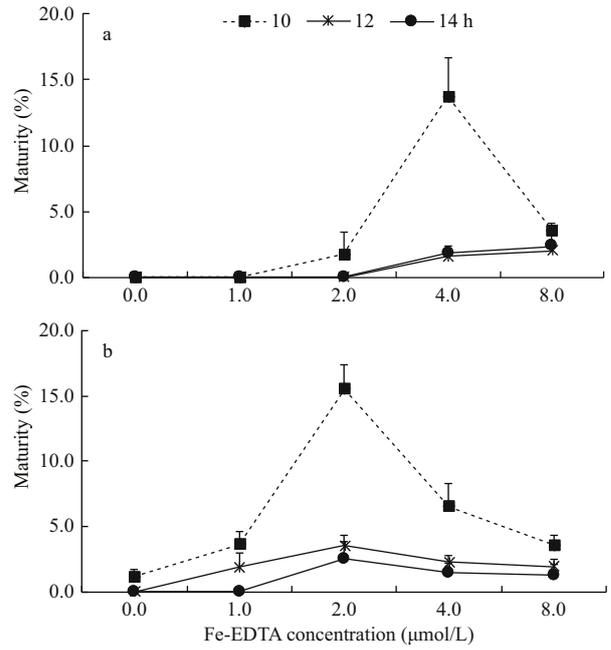
**Table 2** Analysis of variance (two-way ANOVA) for the effects of Fe-EDTA concentration and daylength on the growth of female gametophytes of *Eisenia bicyclis* and *Ecklonia cava*

Factors	<i>Eisenia bicyclis</i>				<i>Ecklonia cava</i>		
	df	MS	F	P	MS	F	P
Fe-EDTA	4	2.51	7.87	<0.01	22.99	64.63	<0.001
Daylength	2	17.81	55.76	<0.01	13.16	37.02	<0.001
Interaction	8	0.18	0.57	0.79	1.73	4.85	<0.001
Error	30	0.32			0.36		

Tukey test (P=0.05)

Fe-EDTA	0<2<4<8, 0=1, 1=2	0=1=4<8, 1=2, 2<0<4<8
Daylength	10<12=14	10<12<14

at Fe-EDTA concentration of 4 μmol/L was significantly higher than those at other four Fe-EDTA concentrations (Fig.4a; Table 3). In contrast, the maturity of female gametophytes of *Ec. cava* at Fe-EDTA concentration of 2 μmol/L was significantly higher than those at other four Fe-EDTA concentrations. For each species, the maturity of female gametophytes at daylength of 10 h was significantly higher than that at daylength of 12 h or 14 h. Significant interactions were observed between



**Fig.4** Maturity (%) of female gametophytes of *Eisenia bicyclis* (a) and *Ecklonia cava* (b) cultured for 10 days at five Fe-EDTA concentrations and three daylengths  
Data represents mean±SE (n=3 replicates).

**Table 3** Analysis of variance (two-way ANOVA) for the effects of Fe-EDTA concentration and daylength on the maturity of female gametophytes of *Eisenia bicyclis* and *Ecklonia cava*

Factors	<i>Eisenia bicyclis</i>				<i>Ecklonia cava</i>		
	df	MS	F	P	MS	F	P
Fe-EDTA	4	55.81	21.56	<0.001	59.59	20.63	<0.001
Daylength	2	44.16	17.13	<0.001	111.09	38.45	<0.001
Interaction	8	25.21	9.78	<0.001	22.34	7.73	<0.001
Error	30	2.58			2.89		

Tukey test (P=0.05)

Fe-EDTA	0=1=2<8<4	0=1=8, 1=4=8<2, 0<4<2
Daylength	12=14<10	12=14<10

Fe-EDTA concentration and daylength for both species.

#### 4 DISCUSSION

*Ei. bicyclis* and *Ec. cava* clearly have different geographical distribution ranges along the coast of Korea. Therefore, a distinct difference in their temperature requirements was anticipated. In the present study, optimal temperatures for their growth were approximately 15–20°C for gametophytes of *Ei. bicyclis* and approximately 20–25°C for those of *Ec. cava*. Compared to *Ei. bicyclis* gametophytes,

*Ec. cava* gametophytes grew better at higher temperatures. The growth of *Ei. bicyclis* gametophyte was inhibited at a temperature of 25°C. These results indicate that gametophytes of *Ec. cava* have better adaptation to high temperature than those of *Ei. bicyclis*. Warm temperature adapted gametophyte stage might be a major factor that enables survival of *Ec. cava* in warmer waters of Korea. Moreover, it has been reported that young sporophytes of *Ec. cava* have greater survival rate at high temperature than those of *Ei. bicyclis* (Arakawa et al., 2013). Therefore, greater tolerance to high temperatures is considered another important factor that determines the wider distribution of *Ec. cava* than that of *Ei. bicyclis*. A similar finding has been reported previously, showing that distinct differences in temperature required for growth of young sporophytes between *Undaria pinnatifida* and *Undaria undarioides* partially contribute to their different distribution patterns (Morita et al., 2003b).

In regional populations of Korea, the optimal temperature for growth of *Ec. cava* gametophyte has been reported to be 20°C at Jeju Island (Kang and Chung, 2015) and 25°C at Jindo and Shinan (Oh et al., 2015; and this study). Since *Ec. cava* has a wide geographical distribution around the coast of Korea, it is most likely that temperature ecotypes exist in this species, as indicated by Breeman and Pakker (1994). A similar phenomenon has also been reported for *Ecklonia radiata* from New Zealand by Novaczek (1984), showing that the temperature tolerance range for the growth and reproduction of *Ec. radiata* gametophytes vary between two geographically separated populations. Plants from warmer Goat Island Bay grew at 9.3–25°C and reproduced at 9.3–24°C, whereas plants from cooler Houghton Bay grew at 8–24°C and reproduced up to 15°C (Novaczek, 1984). On the other hand, although *Ei. bicyclis* is limited along the coasts of Ulleungdo and Dokdo Islands, it has a wide distribution range in Japanese waters (Yoshida, 1998). This also indicates the possibility of ecotype existence. This hypothesis is supported by previous results showing that *Ei. bicyclis* gametophytes from northern Japan, exhibit maximal growth at 20°C (Cao et al., 2014), in agreement with results of the present study. However, optimal growth temperature for *Ei. bicyclis* gametophytes from Miyagi and Chiba, Japan is 24°C (Taniguchi and Akiyama, 1982; Baba, 2010). Therefore, further researches should be carried out to clarify the ecotypic differentiation of these two species.

Vertical distribution of subtidal seaweeds is closely

related to the quantity of light that penetrates into the habitat (Lobban and Harrison, 1994). On the coasts of Ulleungdo and Dokdo Islands, habitat depth of *Ec. cava* ranges from 2 m to 25 m while that of *Ei. bicyclis* ranges from 2 m to 10 m in depth (Kang et al., 2001; Choi et al., 2009), indicating that *Ei. bicyclis* may prefer shallower waters with higher light intensity. This hypothesis is supported by a previous finding showing that compensation light intensity and critical light intensity of juvenile sporophytes of *Ei. bicyclis* are higher than those of *Ec. cava* (Maegawa et al., 1988). They have suggested that light intensity might be an important factor that determines the difference in the lower limit of vertical distribution of these two species. However, in the present study, gametophytes of both species showed the best growth at the low light intensity of 20  $\mu\text{mol photons}/(\text{m}^2\cdot\text{s})$ , consistent with the optimum light intensity of 10–20  $\mu\text{mol photons}/(\text{m}^2\cdot\text{s})$  for *Ei. bicyclis* plants collected from northern Japan (Taniguchi and Akiyama, 1982). These phenomena may be related to their adaptation to a low-light condition resulting from light absorption by blades in the canopy and water column (Maegawa et al., 1988). Additionally, at low-light conditions with a light intensity of 10 and 20  $\mu\text{mol photons}/(\text{m}^2\cdot\text{s})$ , the growth rates of gametophytes of *Ec. cava* were significantly higher than those of *Ei. bicyclis* under the same temperature of 20°C (Fig.2), indicating that *Ec. cava* could adapt better to low-light condition than *Ei. bicyclis*. This advantage might have partially contributed to the wider vertical distribution of *Ec. cava* along the coasts of Ulleungdo and Dokdo Islands.

In the present study, the growth of gametophytes of *Ei. bicyclis* and *Ec. cava* was significantly enhanced by increasing Fe-EDTA concentrations during culture experiments. Similarly, *S. japonica* exhibited greater growth when Fe ion concentration was increased in the seawater and tissue (Matsunaga et al., 1994). The growth of germlings and juveniles of different *Sargassum* species is also positively correlated with an increase in Fe-EDTA concentration (Nagai et al., 2014; Miki et al., 2016). It should be noted that the RGR of *Ei. bicyclis* gametophyte was increased more than 3 times from 0 to 8  $\mu\text{mol/L}$ , whereas that of *Ec. cava* gametophyte was increased approximately 1.3 times. This result indicate that gametophytes of *Ei. bicyclis* have higher sensitivity to the increase of Fe ion concentration than those of *Ec. cava*.

The maturity of gametophytes of *Ei. bicyclis* and *Ec. cava* was also greatly enhanced by increasing Fe

ion concentration. Similar results have been reported previously, showing that significantly higher maturity percentages are found in gametophytes of *S. angustata* and *S. japonica* at Fe ion concentration of 2 mg/L compared to those of the control group without Fe ion addition (Motomura and Sakai, 1981, 1984). However, in the present study, these two species showed different optimal Fe ion concentration for gametophyte maturity, with *Ei. bicyclis* having higher Fe ion requirement than *Ec. cava*. Recently, Iwai et al. (2015) suggested that *S. japonica* forests could be restored by adding a small amount of Fe ion (1  $\mu$ mol/L). Therefore, the addition of Fe ion might be useful for recovering forests of these two species in barren grounds, especially for *Ei. bicyclis*.

## 5 CONCLUSION

In conclusion, both temperature and light intensity had significant effects on the growth of female gametophytes of *Ei. bicyclis* and *Ec. cava*. Compared to *Ei. bicyclis*, *Ec. cava* showed greater adaptation to warmer temperatures. This might be a major factor that contributes to its wider geographical distribution in Korean waters. In addition, the growth and maturation of gametophytes of these two species were significantly affected by day length and enhanced by Fe ion addition. However, they showed different sensitivities to Fe ion concentration for maturation. Since there is a strong need for restoration of kelp forests in different waters, these data may contribute valuable information to obtain optimal results. Morita et al. (2003a) have reported that the difference in maturation temperature range of gametophytes is a major factor determining the distribution of two *Undaria* species along the Japanese coast. Therefore, further study is needed to investigate temperature characteristics for gametophyte maturation of *Ei. bicyclis* and *Ec. cava*.

## 6 DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## 7 ACKNOWLEDGMENT

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