

## Article

# Geographic Differentiation of Morphological Characteristics in the Brown Seaweed *Sargassum thunbergii* along the Korean Coast: A Response to Local Environmental Conditions

Sangil Kim <sup>1</sup> , Sun Kyeong Choi <sup>2</sup>, Seohyeon Van <sup>1</sup>, Seong Taek Kim <sup>3</sup>, Yun Hee Kang <sup>4,\*</sup> and Sang Rul Park <sup>2,\*</sup><sup>1</sup> Ocean Climate and Ecology Research Division, National Institute of Fisheries Science, Busan 46083, Korea; sikim10@korea.kr (S.K.); shvan@korea.kr (S.V.)<sup>2</sup> Estuarine and Coastal Ecology Laboratory, Department of Marine Life Sciences, Jeju National University, Jeju 63243, Korea; choisk@jejunu.ac.kr<sup>3</sup> B&G Ecotech Co., Ltd., Jinju 52818, Korea; kst73@naver.com<sup>4</sup> Department of Earth and Marine Sciences, Jeju National University, Jeju 63243, Korea

\* Correspondence: unirang78@gmail.com (Y.H.K.); srpark@jejunu.ac.kr (S.R.P.); Tel.: +82-64-754-3425 (S.R.P.)

**Abstract:** Intraspecific variation in morphology is widespread among seaweed species in different habitats. We examined the morphological variation in *Sargassum thunbergii* involving diverse environmental factors. We quantified 16 morphological characteristics on 15 rocky intertidal shores in Korea. A cluster analysis based on morphology identified three groups. Group M1 comprised populations on the northern part of the east coast, where the thalli was short and thick, with large leaf and air-vesicle. Group M3 consisted of populations on the west coast exclusively separated from other populations, with short, slender and sparsely branched thalli. Group M2 comprised populations on the southern part of the east coast and on the south coast (including Jeju Island), with longest thalli and lateral branches. Principal coordinate analyses showed that group M1 and M3 were mostly influenced by strong wave action and large tidal amplitudes, respectively. Group M2 were under the influence of warm temperatures and high irradiance. Biota-environment matching analysis showed that the morphology is affected by combinations of different local environmental factors and also that tidal condition is important as a single variable, suggesting that morphology of *S. thunbergii* reflects and adapts to local environmental conditions.

**Keywords:** *Sargassum thunbergii*; morphological variability; seaweed morphology; multiple environmental factors; intertidal zone



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## 1. Introduction

The populations of plant species in different habitat show alternative morphological forms. The degree of intraspecies morphological differences is dependent on dispersal distances and gene flow [1]. Considering that their life history traits were closely related with morphology, morphological variability has important ecological and physiological implications for the plant themselves and the organisms associated with them [2–5]. Seaweeds having broad habitat ranges, from intertidal to subtidal zones, usually show high intraspecific morphological variations [6]. Such morphological variability is believed to extend selective advantages to individuals inhabiting various environmental conditions [7–10]. For example, large brown seaweeds such as *Laminaria longicruris* and *Eisenia arborea* have narrow and strap-like blades to enhance their survival and fitness under wave-exposed condition [8,11]. Thus, the existing morphological characteristics in seaweeds would reflect local environmental conditions where they inhabit.

Seaweed morphology is influenced by many environmental factors, including season [12], depth [13,14], nutrient availability [2,15], wave action [16], irradiance [17,18], temperature and salinity [19–21]. For example, *Ulva fenestrata* shows the optimal thallus

growth at high irradiance and low temperature [17] and seaweeds growing in wave-exposed sites (e.g., *Codium fragile*, *Fucus* spp. and *Ecklonia radiata*) are often much smaller than those growing in sheltered waters [16,22–24]. Similarly, *Saccharina latissima* has narrow blade in hyposaline condition [19]. Due to the relationships between morphological development and extrinsic conditions, some seaweed species can be used as environmental indicators [25,26]. Hence, improved understanding of the impacts of environmental conditions on seaweed morphology can be an important element in investigations of coastal ecological health [27,28].

Morphological variability in seaweed results from the interactions of the multifactors [17,18]. Wave action is well known as a major physical factor that influence thallus size and intraspecific morphological variability in seaweed because many studies have focused on the effects of wave action [29]. Relatively few have investigated the influence of other abiotic factors, such as salinity, desiccation, nutrient concentration and temperature, which affect morphology indirectly by altering physiological responses to stressors [2,21,30,31]. Furthermore, biotic factors, biomass losses and defenses induced by grazing pressure can affect morphological variability [27,30]. Although morphological variation is affected by the complex interactions of multiple stressors [23,32], many investigations have regarded only one or two factors. A better understanding of the relationships between morphology and environment requires a more comprehensive approach that considers multiple factors simultaneously.

The genus *Sargassum* C. Agardh is one of the most species-rich genera among the Fucales, Phaeophyceae [33]. *Sargassum thunbergii*, an ecologically and economically important brown seaweed [34], is widely distributed from middle to lower intertidal zones in the coastal areas of Northeast Asia including Korea, China and Japan [35–37]. This species has high morphological variability and adjusts its growth form in accordance with varying levels of local environmental conditions [34,38–40]. *S. thunbergii* provides habitat, shelter and nursery areas for a wide variety of flora and fauna [41]. This species is especially an important feed source for abalone and the rapidly developing holothurian aquaculture. Additionally, *S. thunbergii* is a major source of bioactive components such as alginate and pharmaceutical products and widely used in biosorptions of trace metal ions [42–44]. Lately, due to large biomass and high productivity, this species has been proposed as an appropriate candidate species to artificially restore large seaweed forests in the intertidal zone in China [45]. The technology for commercial cultivation of *S. thunbergii* has been developed to meet various types of demand [34]. However, ecological and physiological information available for cultivation, conservation and restoration of *S. thunbergii* is still lacking [41,46,47], even though several studies have been conducted on phylogenetic or population genetic diversity [48–50]. In particular, there have been almost no attempts to identify environmental factors affecting intraspecific morphological variation of *S. thunbergii*.

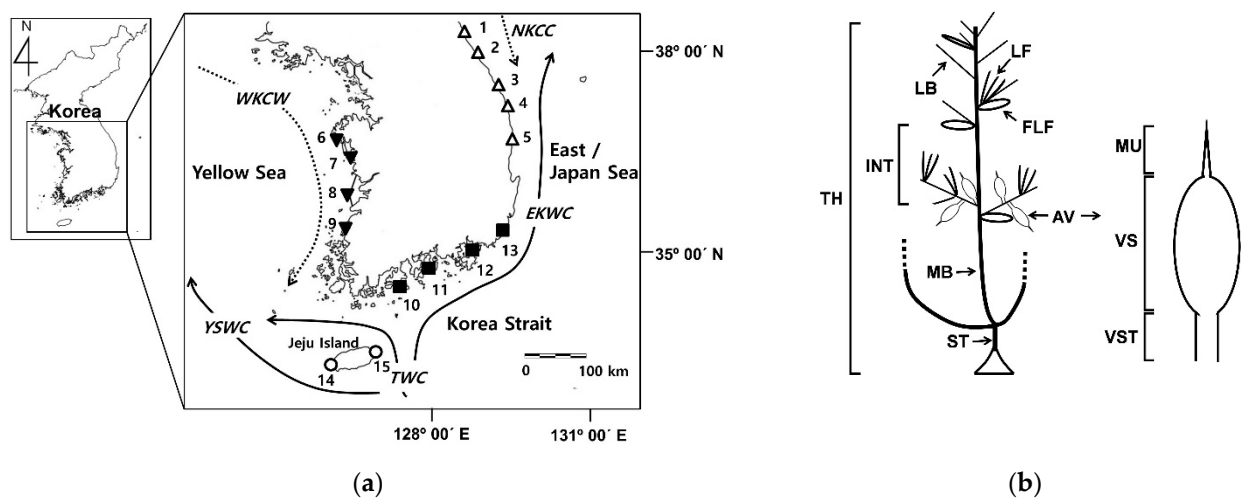
Understanding how morphological traits of seaweed respond to varying environmental factors is important in making management policy for its conservation, restoration and aquaculture. In this study, we hypothesized that morphological differences among *S. thunbergii* populations occur along the Korean coast depending on local environmental conditions. The objectives of this study were (1) to identify the morphological differences among *S. thunbergii* populations grown on Korean coasts and (2) to examine the determinant of their morphology through quantifying the relationship between morphology of *S. thunbergii* and local environmental factors.

## 2. Materials and Methods

### 2.1. Study Area

The present study was conducted at 15 study sites along the coasts of South Korea including Jeju Island (Figure 1a). The eastern coast of Korea is exposed to high wave action and its coastline is simple and monotonous. The tidal amplitude is very small (<30 cm) and seaweed habitats are well developed. The southern part on the eastern coast is affected by

the East Korean Warm Current (EKWC), which originates in the Tsushima Warm Current, while the northern part is influenced by the North Korea Cold Current. The western coast of Korea, which faces the Yellow Sea, is a typical drowned valley shoreline (muddy flats with high turbidity) mostly dominated by macrotidal (tidal amplitudes reaching ca. 9 m). As the West Korea Coastal Current flows around the western coast of Korea, water temperature and salinity are relatively low. The southern coast of Korea has many semi-enclosed bays with moderate wave exposure and is mesotidal, with semi-diurnal tidal range of 1–3 m. Tsushima Warm Current, a branch of the Kuroshio Current, transports warm waters to the southern sea of Korea and is divided to the Yellow Sea Warm Current and the East Korean Warm Current, which affects the southwestern and the southeastern coast of Korea, respectively. Jeju Island, located in the southern sea of Korea, is the largest island in Korea. The climate of Jeju Island is strongly influenced by the Tsushima Warm Current flowing around the island. The tidal regime is similar to that of the southern coast of Korea.



**Figure 1.** Study sites (a) and schematic diagram of *Sargassum thunbergii* (b); (a) Study sites and oceanic currents around Korea. Eastern coast ( $\Delta$ ): 1. Sokcho (SO), 2. Jumunjin (JM), 3. Samcheok (SC), 4. Jukbyeon (JB), 5. Yeongdeok (YD); western coast ( $\blacktriangledown$ ): 6. Taeon (TA), 7. Daecheon (DC), 8. Sinsido Island (SI), 9. Yeonggwang (YG); southern coast ( $\blacksquare$ ): 10. Goheung (GH), 11. Namhae (NH), 12. Geojaedo Island (GJ), 13. Busan (BS); Jeju Island ( $\circ$ ): 14. Gosan (GS), 15. Seongsan (SS). Solid lines indicate warm currents. The Yellow Sea Warm Current (YSWC) and the East Korean Warm Current (EKWC) stem from the Tsushima Warm Current (TWC), which originates in the Kuroshio Warm Current. Dashed lines indicate cold currents: the West Korea Cold Water (WKCW) and the North Korea Cold Current (NKCC) (modified from Gong et al. [51]); (b) Schematic diagram describing the morphology of *S. thunbergii*. Thallus height (TH); main branch (MB); stipe (ST); lateral branch (LB); internode interval (INT); leaf (LF); fulcrant leaf (FLF); air-vesicle (AV); mucro (MU); vesicle (VS); stalk of vesicle (VST).

Fifteen sites where *S. thunbergii* dominated in the rocky shores were selected according to [40]; the distance among sampling sites was more than 40 km (Figure 1a). The eastern sites (site 1–5) were on steep shores subjected to strong wave action. Most *S. thunbergii* populations were permanently submerged even during low tide. The northern and the southern parts on the eastern coasts are affected by cold (NKCC) and warm (EKWC) waters, respectively. Site 3 is simultaneously affected by both NKCC and EKWC. The western sites (site 6–9) have very turbid waters and broad expanses of flat and rocky substratum. During low tide periods, *S. thunbergii* populations are exposed to protracted aerial exposure. The southern sites (site 10–13) and two sites (site 14 and 15) on Jeju Island have moderate wave exposure and tidal ranges. *S. thunbergii* populations in these sites are distributed from middle to lower rocky intertidal habitats with gentle slopes and thus, the populations on the southern coasts are periodically exposed to air during low tide.

## 2.2. Environmental Parameters

Air temperature and sea surface temperature ( $^{\circ}\text{C}$ ), maximum wave height (m), daily incident photon irradiance ( $\text{mol photons m}^{-2} \text{ d}^{-1}$ ) and salinity data were obtained from the Korean Ocean Observing and Forecasting System (KOOFS, [sms.khoa.go.kr/koofs](http://sms.khoa.go.kr/koofs)) and the National Climate Data Service System (NCDSS, [sts.kma.go.kr](http://sts.kma.go.kr)), where daily data were assembled from tidal stations, marine buoys and meteorological observatories at each study site for the last 20 years (1992–2011). Air temperature, sea surface temperature and salinity were measured every 30 or 60 min depending on those observation systems and then averaged daily. Maximum wave height averaged daily the highest wave heights which were calculated at 30 or 60 min intervals from data logged every 0.5 or 1 s. Accumulated daily solar irradiance ( $\text{MJ m}^{-2}$ ) obtained from NCDSS converted to the daily incident photon flux density ( $\text{mol photons m}^{-2} \text{ d}^{-1}$ ) [52]. However, we could not obtain a full set of environmental data at sites 1, 4, 7, 10 and 14. These sites were excluded data analyses for the relationship between morphological variation and environments. The magnitude of tidal range were classified into three categories of dominant water oscillation: category 1, microtidal system (the east coast; generally mixed diurnal or semi-diurnal tides); category 2, mesotidal system (the south coast and Jeju Island; generally semi-diurnal tides); category 3, macrotidal system (the west coast; generally semi-diurnal tides).

## 2.3. Sampling Design and Morphological Measurements

*Sargassum thunbergii* was identified based on morphological characters described by previous literature and their growth form was adjusted in accordance with various local environmental conditions [38–40]. The species also has a high seasonal variation in morphology. In particular, juveniles and subadult plants are morphologically immature during winter, for example, incomplete development of lateral branch and modular organs (leaf, air-vesicle and receptacle) because they are not yet fully grown. For summer and fall, they shed their modular organs and branches due to thallus decay after sexual reproduction and therefore, their morphology is complete and intact during spring season. To measure clearly morphological responses to local conditions and prevent taxonomic misidentification, we haphazardly collected 20 fully grown individuals with all modular organs at each site during spring period, late April–early June 2011 (Figure A1) and 6–16 individuals were used in analysis. The samples were fixed immediately in a 5–10% formalin-seawater solution in the field, transported to the laboratory and processed immediately. Thallus height, main branch diameter, the length and diameter of stipe and longest lateral branch, internode interval, the length, width and thickness of leaf, the length, width and volume of air-vesicle and the length of stalk and mucro were measured to the nearest 0.1 mm using digital calipers as shown in Figure 1b.

## 2.4. Data Analysis

Hierarchical cluster analysis (with group-average linking) and Principal Coordinates Analysis (PCoA) were carried out to identify morphological dissimilarity among the sites; the PCoA ordination plot included morphological vectors. These were based on Euclidean distance with square root-transformed and normalized data because each of the morphological measurements were not on the same scale. Significant differences among the groups formed by cluster analysis were tested by Similarity Profile (SIMPROF). To examine the relationship between morphological characteristics and environmental parameters, BIO-ENV (biota-environment) procedure was applied: this procedure explains the best subset of environmental variables correlated with biological variables [53]. For example, Umanzor et al. [54] demonstrated that the attenuation of irradiance and desiccation stress among the several experimental treatments they performed were best factors to be correlated with the abundance of macroinvertebrates. To use BIO-ENV, the two matrices for environment and morphology must exactly refer to a common set of samples (sampling site). We did not have, however, environmental data of all study sites as mentioned in Section 2.2. BIO-ENV were carried out using the morphological data of the study sites

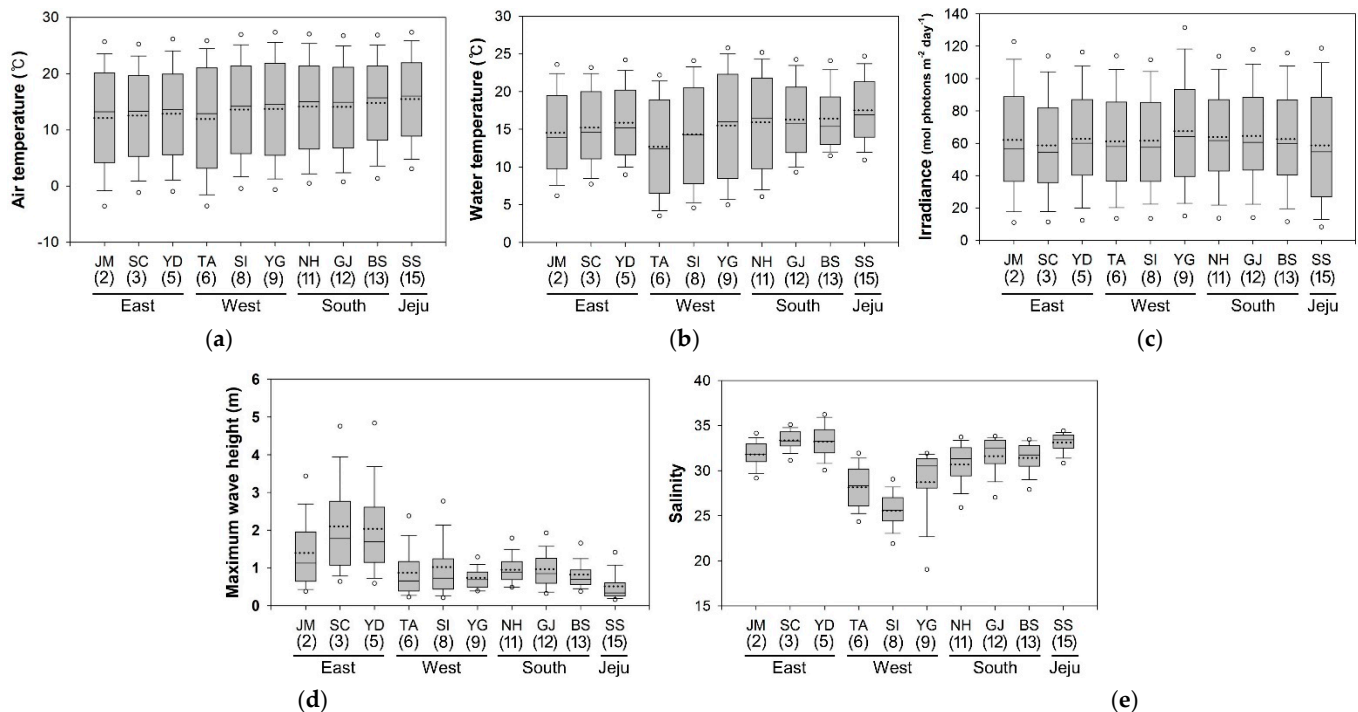
corresponding to where environmental variables were obtained and PCoA ordination plot was also modified accordingly. Here, as superimposing environmental vectors on the modified PCoA ordination plot, we explained correlations among environmental variables, morphological groups and the modified PCoA axes. Multivariate analyses were performed with PRIMER v6 and PERMANOVA+ software (PRIMER-e, Auckland, New Zealand) [53,55].

Significant differences in each of the morphological measurements among the group formed by cluster analysis were tested using a one-way ANOVA. Morphological data were tested for normality and homogeneity of variance to the assumptions of parametric statistics using Shapiro–Wilk’s and Levene’s tests, respectively. If these assumptions were not met, data were  $\log(x + 1)$  transformed prior to analysis. When a significant difference was observed among variables, the means were examined using Student–Newman–Keuls (SNK) tests to determine where the significant difference occurred. Statistical significance was set at  $\alpha < 0.05$ . All values are presented as means  $\pm$  SE (standard error) and ANOVA were conducted using SPSS software (version 20.0, IBM SPSS Statistics, New York, NY, USA).

### 3. Results

#### 3.1. Environmental Parameters

Environmental conditions varied with 10 sampling sites where full sets of data were obtained (Figure 2). The overall air and water temperatures were higher at sampling sites located at southern coasts (including Jeju Island) and lower at sites located at western and eastern coasts (Figure 2a,b). The surface photon irradiance was slightly higher at the southern sites than at other sites (Figure 2c). The wave heights were apparently higher at sites on the east coast than in other regions (Figure 2d). The salinity was lower off the west coast than at other sites (Figure 2e).



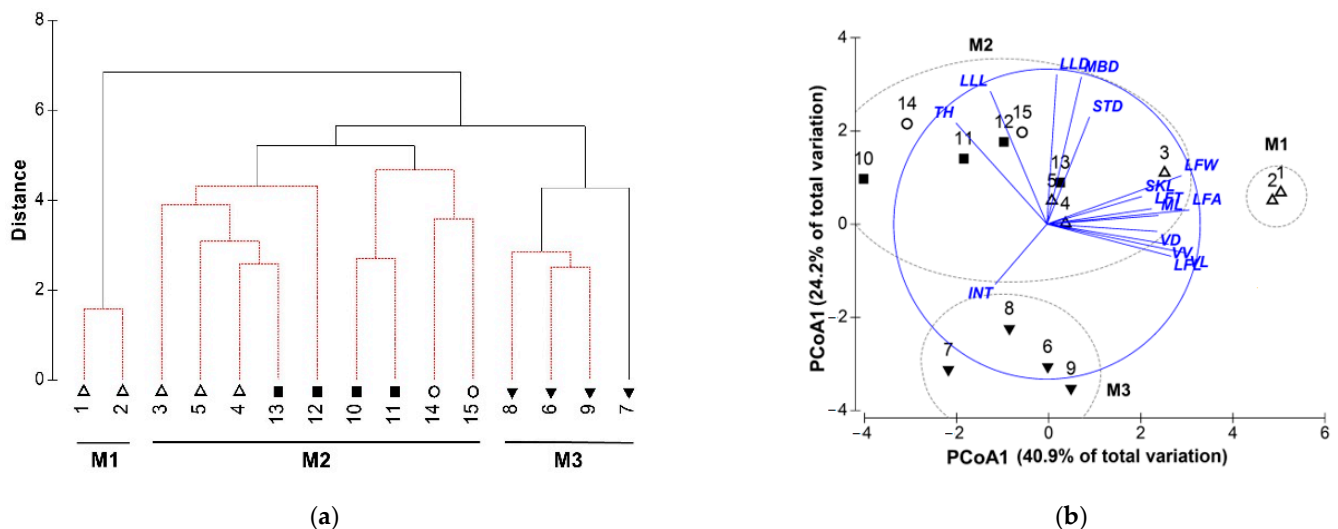
**Figure 2.** Environmental parameters in the study sites: (a) Air temperature; (b) water temperature; (c) incident irradiance; (d) maximum wave height; (e) salinity. Open circles are 5th and 95th percentile; solid line and dotted line indicate median value and mean value, respectively.

#### 3.2. Morphological Variations

Morphological characteristics of *Sargassum thunbergii* were divided into five significant groups by the hierarchical cluster analysis with SIMPROF test ( $\pi = 0.949$ ,  $p = 0.001$ ,



Figure 3a). Site 1 and 2, the northern part on the eastern coasts, were independently separated from other sites (we denominated them group M1). The western sites also formed an exclusive group although they were divided into two groups, site 7 and site 6–9. When SIMPROF test was independently carried out on a subset of the western sites only, they (site 7 and site 6–9) did not show significant difference ( $\pi = 0.043$ ,  $p = 0.89$ ): it would be reasonable to consider the western sites as virtually a single group (M3). The southern sites including Jeju Island and the remains of eastern coasts were significantly split into two groups. SIMPROF test with a subset of sites of these two groups, however, showed nonsignificant differences ( $\pi = 0.223$ ,  $p = 0.7$ ) and therefore, they totally would be a single group (M2). Consequently, morphological characteristics can be grouped into three clusters by an arbitrary distance level of 5.5 (Figure 3b).



**Figure 3.** Hierarchical cluster dendrogram (a) and PCoA ordination plot (b) based on Euclidean distance: (a) Red dotted lines represent no significant differences between sites by SIMPROF test; (b) PCoA ordination plot showed that the study sites can be grouped into three clusters (M1–M3) at an arbitrary distance level of 5.5. Correlations between morphological variables and PCoA axes are represented by superimposed vectors (Pearson correlation,  $r > 0.5$ ) in (b). Sampling sites are indicated by symbols and numbers, which are provided in Figure 1a. STD (stipe diameter); TH (thallus height); LLL (length of longest lateral branch); LLD (diameter of longest lateral branch); MBD (main branch diameter); INT (internode interval); LFL (leaf length); LFW (leaf width); LFT (leaf thickness); LFA (leaf area); VL (vesicle length); VD (vesicle diameter); VV (vesicle volume); SKL (stalk length of vesicle); MU (mucro length).

Morphological characteristics were significantly different among groups M1, M2 and M3 (Table 1). Thallus height and longest lateral branch length were significantly longer in group M2 than in the other groups. The smallest diameters of main and lateral branch occurred in group M3. Stipe diameters were larger in groups M1 and M2 than in group M3. Stipe diameters were significantly different among the groups, but stipe lengths were not. The internode interval was the longest in group M3, meaning sparsely branched. Leaf- and vesicle-related measurements were significantly larger in group M1 than in the other groups. The PCoA plot summarized these findings well (Figure 3b). The first two axes of the PCoA explained 65.1% of the total variation (PCoA1: 40.9%; PCoA2: 24.2%). The axis of PCoA1 was positively correlated with leaf- (LFL:  $r = 0.810$ , LFW:  $r = 0.878$ , LFT:  $r = 0.684$ , LFA:  $r = 0.929$ ) and vesicle-related traits (VL:  $r = 0.912$ , VD:  $r = 0.718$ , VV:  $r = 0.794$ , SKL:  $r = 0.620$ , ML:  $r = 0.727$ ). Group M1 (with large values for leaf and vesicle) was strongly correlated with vectors related to leaf and vesicle and separated from the others along the axis of PCoA1. The axis of PCoA2 was positively correlated with thallus height ( $r = 0.652$ ), longest branch length ( $r = 0.857$ ), branch diameter (MDB:  $r = 0.948$ , LLD:  $r = 0.966$ ) and

stipe diameter ( $r = 0.692$ ). Group M2 (with large values for thallus height and branch, but small leaf and vesicle) was strongly positively correlated with vectors related to thallus height and lateral branch traits. Group M3 (with sparse branching and slender and small thalli) was positively correlated with internode interval.

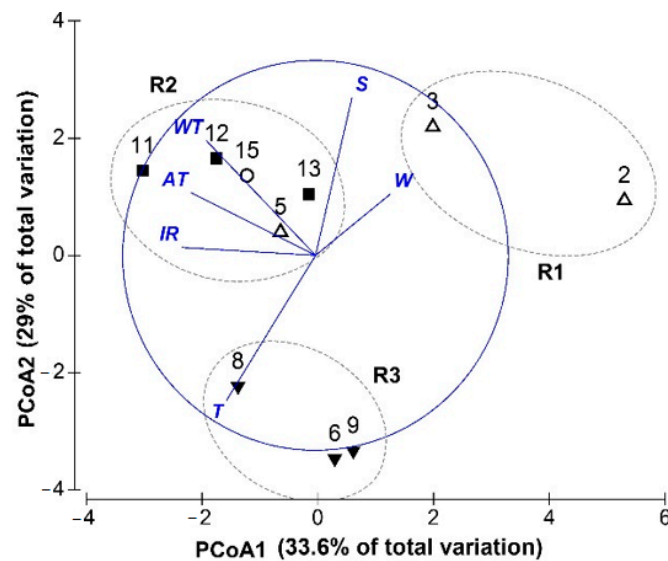
**Table 1.** One-way ANOVA results in morphological measurements of *Sargassum thunbergii* grouped by hierarchical clustering. Data are mean  $\pm$  SE (n = 12–93).

Morphological Characteristics	Code	Group M1	Group M2	Group M3
Thallus				
Thallus height (cm) ***	TH	27.8 $\pm$ 1.1 <sup>c</sup>	68.3 $\pm$ 2.7 <sup>a</sup>	35.2 $\pm$ 1.3 <sup>b</sup>
Main branch diameter (mm) ***	MBD	2.0 $\pm$ 0.1 <sup>a</sup>	2.0 $\pm$ 0.1 <sup>a</sup>	1.2 $\pm$ 0.1 <sup>b</sup>
Stipe length (mm) <sup>NS</sup>	STL	4.1 $\pm$ 0.2 <sup>a</sup>	3.7 $\pm$ 0.2 <sup>a</sup>	3.5 $\pm$ 0.2 <sup>a</sup>
Stipe diameter (mm) ***	STD	6.6 $\pm$ 0.3 <sup>a</sup>	4.9 $\pm$ 0.2 <sup>b</sup>	3.2 $\pm$ 0.1 <sup>c</sup>
Longest lateral branch length (cm) ***	LLL	4.4 $\pm$ 0.4 <sup>b</sup>	9.8 $\pm$ 0.5 <sup>a</sup>	3.2 $\pm$ 0.1 <sup>c</sup>
Longest lateral branch diameter (mm) ***	LLD	1.1 $\pm$ 0.0 <sup>b</sup>	1.2 $\pm$ 0.0 <sup>a</sup>	0.4 $\pm$ 0.0 <sup>c</sup>
Internode interval (mm) ***	INT	2.0 $\pm$ 0.0 <sup>c</sup>	4.2 $\pm$ 0.2 <sup>b</sup>	4.9 $\pm$ 0.2 <sup>a</sup>
Leaf				
Leaf length (mm) ***	LFL	9.2 $\pm$ 0.2 <sup>a</sup>	6.0 $\pm$ 0.2 <sup>c</sup>	6.7 $\pm$ 0.2 <sup>b</sup>
Leaf width (mm) ***	LFW	0.8 $\pm$ 0.0 <sup>a</sup>	0.5 $\pm$ 0.0 <sup>b</sup>	0.4 $\pm$ 0.0 <sup>c</sup>
Leaf thickness (mm) **	LFT	0.41 $\pm$ 0.0 <sup>a</sup>	0.36 $\pm$ 0.0 <sup>b</sup>	0.34 $\pm$ 0.0 <sup>b</sup>
Leaf area (mm <sup>2</sup> ) ***	LFA	7.2 $\pm$ 0.2 <sup>a</sup>	3.1 $\pm$ 0.2 <sup>b</sup>	2.9 $\pm$ 0.1 <sup>b</sup>
Vesicle				
Air-vesicle length (mm) ***	VL	2.9 $\pm$ 0.1 <sup>a</sup>	2.2 $\pm$ 0.0 <sup>b</sup>	2.4 $\pm$ 0.1 <sup>b</sup>
Air-vesicle diameter (mm) *	VD	1.0 $\pm$ 0.0 <sup>a</sup>	0.9 $\pm$ 0.0 <sup>b</sup>	0.9 $\pm$ 0.0 <sup>b</sup>
Air-vesicle volume (mm <sup>3</sup> ) ***	VV	6.7 $\pm$ 0.4 <sup>a</sup>	4.1 $\pm$ 0.2 <sup>b</sup>	4.6 $\pm$ 0.4 <sup>b</sup>
Stalk length (mm) ***	SKL	3.1 $\pm$ 0.1 <sup>a</sup>	2.4 $\pm$ 0.1 <sup>b</sup>	2.2 $\pm$ 0.1 <sup>b</sup>
Mucro length (mm) ***	ML	1.3 $\pm$ 0.1 <sup>a</sup>	0.9 $\pm$ 0.0 <sup>b</sup>	1.0 $\pm$ 0.1 <sup>b</sup>

Superscript of same small letter indicates no significant difference in morphological parameters among groups; \*:  $p < 0.05$ ; \*\*:  $p < 0.01$ ; \*\*\*:  $p < 0.001$ ; <sup>NS</sup>: no significance.

### 3.3. Relationships between Morphological Variations and Environments

We found that the morphological characteristics of *S. thunbergii* have been highly correlated with environmental variables (Figure 4 and Table 2). The locations of morphological groups in the modified PCoA plot which were analyzed using the study sites with environmental variables corresponded to the locations of morphological characteristics in Figure 3b (Figure 4). The study sites that clustered in groups R1, R2 and R3 corresponded to those of groups M1, M2 and M3, but site 3 did not fit this pattern. Site 3 belonged to group R1 corresponding to group M1, rather than to group R2 corresponding to group M2. The grouping clearly corresponded to the direction of the overlain vectors for environmental variables (Figure 4). The vector for wave height had a moderately positive correlation with group R1 ( $r = 0.500$ ), which separated this group from the others. The vectors for air temperature, water temperature and irradiance were strongly positively correlated with group R2 ( $r = 0.772$ ,  $r = 0.785$  and  $r = 0.583$ , respectively). The vector for tidal regime and salinity were strongly positively ( $r = 0.878$ ) and negatively ( $r = 0.832$ ) correlated with group R3, respectively.



**Figure 4.** PCoA ordination plot based on Euclidean distances of square root-transformed morphological parameters. Correlations between environmental variables and PCoA axes are represented by vectors superimposed on the PCoA plot (Pearson correlation,  $r > 0.5$ ). Dashed ellipses are groups distinguished at a distance level of 5.5 in the hierarchical clustering analysis. Group R is also a morphological group and we denominated the name R as illustrating the relationship between morphology and environmental variables. AT (air temperature); WT (water temperature); IR (irradiance); W (wave height); S (salinity); T (tidal regime).

**Table 2.** The best results acquired from BIO-ENV analysis of combined environmental variables matching morphology of *Sargassum thunbergii* (Spearman correlation coefficient,  $\rho$ ).

Number of Variables	Best Combinations of Environmental Variables
1	T (0.342), IR (0.339), AT (0.233), WT (0.182), S (0.099), W (0.002)
2	IR+T (0.464) <sup>5</sup> , AT+T (0.397), AT+IR (0.393), WT+T (0.390)
3	AT+IR+T (0.519) <sup>2</sup> , WT+IR+T (0.519) <sup>2</sup> , AT+WT+T (0.440)
4	AT+WT+IR+T (0.556) <sup>1</sup> , AT+S+IR+T (0.446), WT+S+IR+T (0.443)
5	AT+WT+S+IR+T (0.475) <sup>4</sup> , AT+WT+IR+W+T (0.354)

AT (Air temperature); WT (Water temperature); W (Wave height); IR (Irradiance); S (Salinity); T (Tidal regime). Superscript is five best results in numerical order.

The best permutations of combined environmental variables (BIO-ENV analysis) matching to the morphology of *S. thunbergii* are listed in Table 2. The single variable which best explains the site grouping, in manner consistent with the morphological patterns, is tidal regime ( $\rho = 0.342$ ). Tidal regime was always involved in the best five combinations, indicating it is important to shape the morphology of *S. thunbergii* as a single variable. On the other hand, the successful matches of the best five combinations included two or more environmental variables at least. Spearman correlation coefficients were always greater for combinations of multiple variables than for each single variable. Their Spearman correlation coefficients did not show dramatic differences across the permutations. This result indicates that the morphology of *S. thunbergii* is also influenced by combinations of multiple environmental variables simultaneously rather than by a single dominant.

#### 4. Discussion

##### 4.1. Relationships between Morphological Variation and Environments

The morphology of *Sargassum thunbergii* markedly changed around the Korean coast-line. We found the changes were highly correlated with different environmental conditions on the different shores. Seaweed morphologies are often highly plastic. Thalli in



wave-exposed areas are frequently much smaller and tougher than those growing in wave-protected areas [16,22–24]. Additionally, many abiotic and biotic factors, including wave action [24,29], light intensity [17,18], temperature [21], salinity [19,20] and grazing [56,57], directly or indirectly affect the growth and morphology of seaweeds. Although these factors interactively affect algal morphology, few studies have examined the relationships between seaweed morphology and multiple environmental factors [58]. Our results showed that determining the morphology of *S. thunbergii* was linked in a complex manner with different local environmental conditions.

Group R3 (corresponding to group M3) occurred exclusively on the Korean west coast and their morphological traits were obviously distinct from the other groups. Thalli in this group had short and slender branches and long internode intervals (Figure 3b and Table 1); hence, they were small, soft in texture and sparsely branched thalli. Large tidal amplitude was a best factor grouping the sampling sites as a single variable, which exclusively separated group R3 from other groups (Figure 4 and Table 2). The west coast of Korea has calm waters, but the large tidal amplitudes and a very gentle slope prolong the aerial exposure times of intertidal creatures. During prolonged atmospheric exposure, thalli are exposed to intense stresses, including desiccation, strong solar radiation, mineral nutrient starvation and extreme fluctuations in salinity and temperature. Yu et al. [45] found that photosynthetic activity of the thalli was reduced by desiccation, heat stress and prolonged aerial exposure, as shown previously for other seaweed species [59–61]. Mueller et al. [58] recently emphasized the role of tidal regime-related stressors in the morphological expression of intertidal seaweeds. The distinctive morphology of group R3 in our study can be explained by the extremely stressful conditions of the macrotidal regime. In addition, many seaweeds produce diminutive thalli in low-salinity waters [62]. As an example, short furoid thalli with narrow stipes are frequently observed in low-salinity intertidal zones [20,31]. In the present study, the morphological characteristics of the members of group R3 (also M3) were negatively correlated with salinity (Figure 4). Thus, our results support that physiological responses to the harsh stresses associated with macrotidal regime likely affected the growth and morphogenesis of *S. thunbergii*.

The morphologies of group R2 (corresponding to M2) were strongly correlated with temperatures and irradiance and weakly correlated with wave height (Figure 4). The study sites in this group ranged from Jeju Island to the southern part of the mainland east coast, where the influences of two warm currents prevail (the TWC and EKWC; see Figure 1b). The intertidal zone of the south coast of Korea is particularly sheltered by the ria configuration of the coastline. Thalli in this group were larger than any other groups: they have long and thick branches and thick stipes (Figure 3b and Table 1). Among all study sites, biomass per thallus should be highest in these southern locations. The thick stipes are able to help supporting heavy thalli [63]. Large forms of *S. thunbergii* often occur in warm and/or sheltered locations [35,38,40], but to date, there has been no direct evidence that the size is related to temperature or shelter. Here, we provide more robust evidence to support this postulation. Many species of seaweeds reach larger sizes or biomass in wave-sheltered waters than in areas exposed to strong hydrodynamic forces (e.g., [11,22,24,64,65]). Growth and survival of seaweed need optimal range of temperature, water motion to allow for nutrient uptake, but not too strong to remove algal thallus, and sufficient light of high levels without photo-inhibition. As identifying the above-mentioned optimal conditions for growth of *S. thunbergii* is not the focus of this study, we do not cover that in detail here. Nevertheless, given our results and distribution of large forms in warm and/or sheltered areas, morphological characteristics of *S. thunbergii* in the southern locations likely result from proper combinations of those environmental factors. Actually, growth of *S. thunbergii* in marine farms increases with temperature and the thallus length reached about 2 m [66]; note the fact that most marine farms are located in sheltered and environmentally suitable places for the maximum growth and survival of the subject organism. These results indicate that strong light, warm temperatures and shelter may promote the formation of large thalli in *S. thunbergii*.

The morphologies of group R1 (corresponding to M1) were correlated with wave height (Figure 4). Specimens of group M1, on the northern part of the east coast, had large leaves and vesicles and their thalli and lateral branch were very short, thick and bushy (Figure 3b and Table 1). Such a morphological form would likely be advantageous in the wave-exposed environments of the northern part of the east coast. For example, thick and flexible stipe and small and thick thallus are very effective in preventing dislodgement and/or breakage from the strong hydrodynamic forces [8,16]. *S. thunbergii* on the east coast have been mostly submerged even during the low tide due to very small tidal amplitude. Thalli on this coast were very dense, which may have caused self-shading. Large air vesicles likely have functional significance in competition for light by providing increased buoyancy to raise thalli towards the surface. Large leaves can also enhance light interception due to maximized leaf surface. Therefore, morphological characteristics of group M1 would be a typical seaweed form in wave-exposed areas.

#### 4.2. Environmental Factors in the Intertidal Zone

Although the sites on the east coast of Korea are subjected to strong wave exposure (Figure 2d and see 2.1 study site), wave height did not completely separate the five sites (site 1–5) from other sites. At first glance, it can make a hasty prediction that morphologies on the east coast (site 1–5) would strongly correlate with wave height. However, the morphologies on this coast fell into two groups, northern part (M1 and R1) and southern part (M2 and R2) according to correlations with different environmental variables (Figures 3b and 4). The site 3 is geographically located between the sites 1–2 and 4–5 on the eastern coast. Interestingly, the same pattern also appeared on the PCoA plots and site 3 belonged to both morphological groups M2 and R1 (Figures 3b and 4). These mean that morphologies of specimens on site 3 have an intermediate form resulting from a combined effect of different environmental variables. Actually, morphological characteristics of both group M1 and M2, or intermediate forms between them were observed at the specimens from site 3. There are two different water masses near site 3, where two currents with different temperatures meet (Figure 1a). Thalli at site 3 were very likely exposed to a complexity of environmental factors, including wave heights, warm and cold water temperatures and fluctuating irradiance. These indicate that combined effects of local environmental variables influenced the morphological grouping although the whole of the Korean east coast is affected by high waves. Our findings are in line with previous studies that demonstrated seaweed morphology in response to local environmental conditions when the degree of wave exposures were similar [23,32].

Many studies have suggested that the degree of wave exposure is the most important determinant of morphology and survival in seaweed (reviewed by Hurd [29]). Water turbulence is likely a principal factor in the subtidal zone, where environmental conditions are stable compared to the intertidal zone, but this is just one of many interacting factors (e.g., irradiance, temperature, nutrients) in the intertidal zone that vary dramatically in space and time [29]. In this study, wave height was only a partially dominant factor in the northern part of the east coast due to locally different environmental variations (Figure 4). Rather, as a single variable, tidal regime was more important (Table 2); it separated exclusively the western coast sites as a whole from other sites (Figure 4). This is probably because tide-induced stresses are greater than wave-induced impacts in intertidal zone [58]. Therefore, tidal condition would be a more important factor shaping morphology of intertidal seaweeds than wave forces at regional scale.

Meanwhile, there are different root causes in the morphological differentiation of seaweeds. For example, dwarf forms in seaweeds have several different causes in response to the harsh environments such as high on the shore [67,68] and tide-induced stress [58] as well as high wave action [16,24]. In this study, although thallus height was significantly shorter in group M1 and M3 than in group M2 (Table 1), the underlying causal factors were different. The short size of thalli in group M1 is linked to strong wave action, while that of thalli in group M3 was linked to tide-induced stress. Members of group M2 had the largest

thallus due to favorable conditions such as temperatures, irradiance and less wave impact. Thus, the morphological differentiation in intertidal seaweeds is controlled by combinations of various local environmental factors rather than by a single dominant effect.

## 5. Conclusions

This study examined morphological variability in intertidal brown seaweed *Sargassum thunbergii* and their relationships with diverse environmental variables at regional scale. Tidal-induced stresses were important as a single variable at regional scale while wave force was a lesser environmental factor which should be considered together with local conditions in intertidal zone. Simultaneously, our results showed morphological differentiation adjusting to locally different environmental conditions, highlighting consideration of multiple environmental variables to examine biological and ecological processes of intertidal seaweeds. Diverse morphologies of *S. thunbergii* resulted from adaptation to different habitat conditions and could provide information about the environments where they inhabit. This study provides valuable information regarding coastal ecological health, restoration and aquaculture using *S. thunbergii*. There are, of course, other factors affecting seaweed morphology (e.g., nutrients, season and grazing) which were not involved in this study. Further studies incorporating them can improve understanding morphological variability of intertidal seaweeds.

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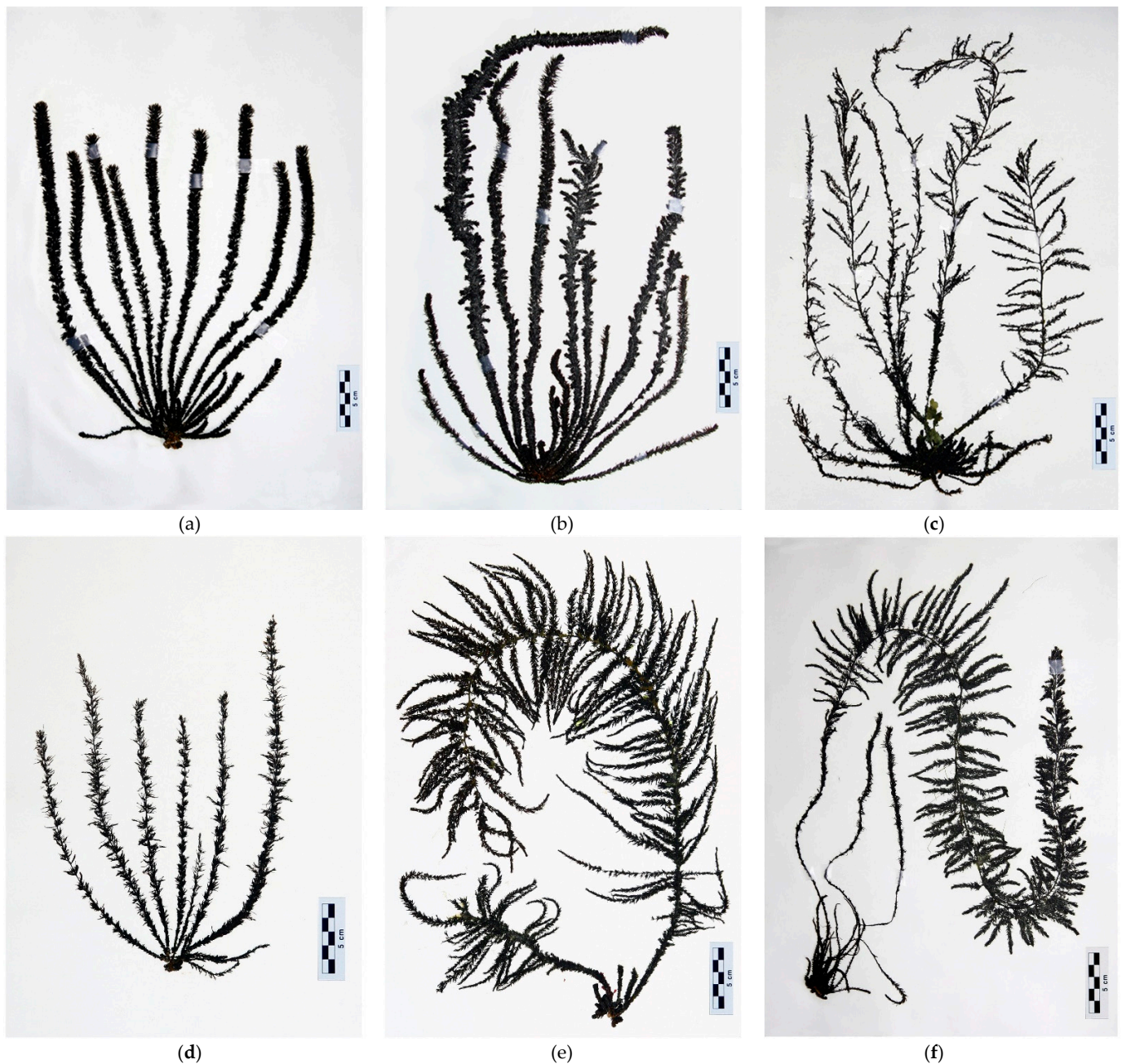
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## Appendix A



**Figure A1.** Photographs of the representative *Sargassum thunbergii* specimens collected from our study sites: (a) The northern part of the east coast (st. 1–2); (b) the mid part of the east coast (st. 3); (c) the southern part of the east coast (st. 4–5); (d) the west coast (st. 6–9); (e) the south coast; (f) Jeju Island. Specimens (c,e,f) are just a part of a whole individual because almost all the large branches have been cut off to make a specimen on the limited paper. Scale bar indicates 5 cm.

## References

1. Slatkin, M. Gene flow and the geographic structure of natural populations. *Science* **1987**, *236*, 787–792. [[CrossRef](#)] [[PubMed](#)]
2. Blanchette, C.A.; Miner, B.G.; Gaines, S.D. Geographic variability in form, size and survival of *Egregia menziesii* around Point Conception, California. *Mar. Ecol. Prog. Ser.* **2002**, *239*, 69–82. [[CrossRef](#)]
3. Fowler-Walker, M.J.; Gillanders, B.M.; Connell, S.D.; Irving, A.D. Patterns of association between canopy-morphology and understory assemblages across temperate Australia. *Estuar. Coast. Shelf. Sci.* **2005**, *63*, 133–141. [[CrossRef](#)]



4. Cacabelos, E.; Olabarria, C.; Incera, M.; Troncoso, J.S. Effects of habitat structure and tidal height on epifaunal assemblages associated with macroalgae. *Estuar. Coast. Shelf. Sci.* **2010**, *89*, 43–52. [\[CrossRef\]](#)
5. Wernberg, T.; Vanderklift, M.A. Contribution of temporal and spatial components to morphological variations in the kelp *Ecklonia* (Laminariales). *J. Phycol.* **2010**, *46*, 153–161. [\[CrossRef\]](#)
6. Littler, M.M.; Littler, D.S. The evolution of thallus form and survival strategies in benthic marine macroalgae: Field and laboratory tests of a functional form model. *Am. Nat.* **1980**, *116*, 25–44. [\[CrossRef\]](#)
7. Duggins, D.O.; Eckman, J.E.; Siddon, C.E.; Klinger, T. Population, morphometric and biomechanical studies of three understory kelps along a hydrodynamic gradient. *Mar. Ecol. Prog. Ser.* **2003**, *265*, 57–76. [\[CrossRef\]](#)
8. Roberson, M.L.; Coyer, J.A. Variation in blade morphology of the kelp *Eisenia arborea*: Incipient speciation due to local water motion? *Mar. Ecol. Prog. Ser.* **2004**, *282*, 115–128. [\[CrossRef\]](#)
9. Benedetti-Cecchi, L.; Bertocci, I.; Vaselli, S.; Maggi, E. Morphological plasticity and variable spatial patterns in different populations of the red alga *Rissoella verrucosa*. *Mar. Ecol. Prog. Ser.* **2006**, *315*, 87–98. [\[CrossRef\]](#)
10. Charrier, B.; Le Bail, A.; de Reviers, B. Plant Proteus: Brown algal morphological plasticity and underlying developmental mechanisms. *Trends Plant. Sci.* **2012**, *17*, 468–477. [\[CrossRef\]](#)
11. Gerard, V.A.; Mann, K.H. Growth and production of *Laminaria longicruris* (Phaeophyta) populations exposed to different intensities of water movement. *J. Phycol.* **1979**, *15*, 33–41. [\[CrossRef\]](#)
12. Falace, A.; Bressan, G. Seasonal variations of *Cystoseira barbata* (Stackhouse) C. Agardh frond architecture. *Hydrobiologia* **2006**, *555*, 193–206. [\[CrossRef\]](#)
13. Engelen, A.H.; Åberg, P.; Olsen, J.L.; Stam, W.T.; Breeman, A.M. Effects of wave exposure and depth on biomass, density and fertility of the fucoid seaweed *Sargassum polyceratum* (Phaeophyta, Sargassaceae). *Eur. J. Phycol.* **2005**, *40*, 149–158. [\[CrossRef\]](#)
14. Stewart, H. Morphological variation and phenotypic plasticity of buoyancy in the macroalga *Turbinaria ornata* across a barrier reef. *Mar. Biol.* **2006**, *149*, 721–730. [\[CrossRef\]](#)
15. Malta, E.-J.; Ferreira, D.G.; Vergara, J.J.; Pérez-Lloréns, L. Nitrogen load and irradiance affect morphology, photosynthesis and growth of *Caulerpa prolifera* (Bryopsidales: Chlorophyta). *Mar. Ecol. Prog. Ser.* **2005**, *298*, 101–114. [\[CrossRef\]](#)
16. D'Amours, O.; Scheibling, R.E. Effect of wave exposure on morphology, attachment strength and survival of the invasive green alga *Codium fragile* ssp. *tomentosoides*. *J. Exp. Mar. Biol. Ecol.* **2007**, *351*, 129–142. [\[CrossRef\]](#)
17. Toth, G.B.; Harrysson, H.; Wahlström, N.; Olsson, J.; Oerbekke, A.; Steinhagen, S.; Kinnby, A.; White, J.; Albers, E.; Edlund, U.; et al. Effects of irradiance, temperature, nutrients, and pCO<sub>2</sub> on the growth and biochemical composition of cultivated *Ulva fenestrata*. *J. Appl. Phycol.* **2020**, *32*, 3243–3254. [\[CrossRef\]](#)
18. Monro, K.; Poore, A.G.B.; Brooks, R. Multivariate selection shapes environment-dependent variation in the clonal morphology of a red seaweed. *Evol. Ecol.* **2007**, *21*, 765–782. [\[CrossRef\]](#)
19. Vettori, D.; Nikora, V.; Biggs, H. Implications of hyposaline stress for seaweed morphology and biomechanics. *Aquat. Bot.* **2020**, *162*, 103188. [\[CrossRef\]](#)
20. Kalvas, A.; Kautsky, L. Morphological variation in *Fucus vesiculosus* populations along temperature and salinity gradients in Iceland. *J. Mar. Biol. Assoc. U. K.* **1998**, *78*, 985–1001. [\[CrossRef\]](#)
21. Kübler, J.E.; Dudgeon, S.R. Temperature dependent change in the complexity of form of *Chondrus crispus* fronds. *J. Exp. Mar. Biol. Ecol.* **1996**, *207*, 15–24. [\[CrossRef\]](#)
22. Blanchette, C.A. Size and survival of intertidal plants in response to wave action: A case study with *Fucus gardneri*. *Ecology* **1997**, *78*, 1563–1578. [\[CrossRef\]](#)
23. Ruuskanen, A.; Bäck, S.; Reitalu, T. A comparison of two cartographic exposure methods using *Fucus vesiculosus* as an indicator. *Mar. Biol.* **1999**, *134*, 139–145. [\[CrossRef\]](#)
24. Fowler-Walker, M.J.; Wernberg, T.; Connell, S.D. Differences in kelp morphology between wave sheltered and exposed localities: Morphologically plastic or fixed traits? *Mar. Biol.* **2006**, *148*, 755–767. [\[CrossRef\]](#)
25. Kilar, J.A.; McLachlan, J. Branching morphology as an indicator of environmental disturbance: Testing the vegetative fragmentation of *Acanthophora spicifera* and the turf morphology of *Laurencia papillosa*. *Aquat. Bot.* **1986**, *24*, 115–130. [\[CrossRef\]](#)
26. Yñiguez, A.T.; McManus, J.W.; Collado-Vides, L. Capturing the dynamics in benthic structures: Environmental effects on morphology in the macroalgal genera *Halimeda* and *Dictyota*. *Mar. Ecol. Prog. Ser.* **2010**, *411*, 17–32. [\[CrossRef\]](#)
27. Miner, B.G.; Sultan, S.E.; Morgan, S.G.; Padilla, D.K.; Relyea, R.A. Ecological consequences of phenotypic plasticity. *Trends Ecol. Evol.* **2005**, *20*, 685–692. [\[CrossRef\]](#)
28. Balata, D.; Piazzzi, L.; Rindi, F. Testing a new classification of morphological functional groups of marine macroalgae for the detection of responses to stress. *Mar. Biol.* **2011**, *158*, 2459–2469. [\[CrossRef\]](#)
29. Hurd, C.L. Water motion, marine macroalgal physiology, and production. *J. Phycol.* **2000**, *36*, 453–472. [\[CrossRef\]](#)
30. Hay, M.E. The functional morphology of turf-forming seaweeds: Persistence in stressful marine habitats. *Ecology* **1981**, *62*, 739–750. [\[CrossRef\]](#)
31. Gylle, A.M.; Nygård, C.A.; Ekelund, N.G.A. Desiccation and salinity effects on marine and brackish *Fucus vesiculosus* L. (Phaeophyceae). *Phycologia* **2009**, *48*, 156–164. [\[CrossRef\]](#)
32. Wernberg, T.; Thomsen, M.S. The effect of wave exposure on the morphology of *Ecklonia radiata*. *Aquat. Bot.* **2005**, *83*, 61–70. [\[CrossRef\]](#)



33. Boundouresque, C.F. Taxonomy and Phylogeny of Unicellular Eukaryotes. In *Environmental Microbiology: Fundamentals and Applications*; Bertrand, J.C., Caumette, P., Lebaron, P., Matheron, R., Normand, P., Sime-Ngando, T., Eds.; Springer: Dordrecht, The Netherlands, 2015; pp. 191–257. [\[CrossRef\]](#)
34. Liu, F.-L.; Li, J.-J.; Liang, Z.-R.; Zhang, Q.-S.; Zhao, F.-J.; Jueterbock, A.; Critchley, A.T.; Morrell, S.L.; Assis, J.; Tang, Y.-Z.; et al. A concise review of the brown seaweed *Sargassum thunbergii*—A knowledge base to inform large-scale cultivation efforts. *J. Appl. Phycol.* **2021**, *33*, 3469–3482. [\[CrossRef\]](#)
35. Umezaki, I. Ecological studies of *Sargassum thunbergii* (Mertens) O. Kuntze in Maizuru Bay, Japan Sea. *Bot. Mag. Tokyo* **1974**, *87*, 285–292. [\[CrossRef\]](#)
36. Zhang, Q.S.; Li, W.; Liu, S.; Pan, J.H. Size-dependence of reproductive allocation of *Sargassum thunbergii* (Sargassaceae, Phaeophyta) in Bohai Bay, China. *Aquat. Bot.* **2009**, *91*, 194–198. [\[CrossRef\]](#)
37. Cho, S.M.; Lee, S.M.; Ko, Y.D.; Mattio, L.; Boo, S.M. Molecular systematic reassessment of *Sargassum* (Fucales, Phaeophyceae) in Korea using four gene regions. *Bot. Mar.* **2012**, *55*, 473–484. [\[CrossRef\]](#)
38. Yendo, K. The Fucaceae of Japan. *J. Coll. Sci. Tokyo Imp. Univ.* **1907**, *21*, 1–174.
39. Okamura, K. *Icones of Japanese Algae*; Kazamashobo: Tokyo, Japan, 1923; Volume V, pp. 6–8.
40. Oak, J.H.; Lee, I.K. Taxonomy of the genus *Sargassum* (Fucales, Phaeophyceae) from Korea I. Subgenus *Bactrophycus* Section *Teretia*. *Algae* **2005**, *20*, 77–90. [\[CrossRef\]](#)
41. Chu, S.H.; Zhang, Q.S.; Liu, S.K.; Tang, Y.Z.; Zhang, S.B.; Lu, Z.C.; Yu, Y.Q. Tolerance of *Sargassum Thunbergii* germlings to thermal, osmotic and desiccation stress. *Aquat. Bot.* **2012**, *96*, 1–6. [\[CrossRef\]](#)
42. Kang, J.Y.; Khan, M.N.A.; Park, N.H.; Cho, J.Y.; Lee, M.C.; Fujii, H.; Hong, Y.K. Antipyretic, analgesic, and anti-inflammatory activities of the seaweed *Sargassum fulvellum* and *Sargassum thunbergii* in mice. *J. Ethnopharmacol.* **2008**, *116*, 187–190. [\[CrossRef\]](#)
43. Kim, J.-A.; Karadeniz, F.; Ahn, B.-N.; Kwon, M.S.; Mun, O.-J.; Bae, M.J.; Seo, Y.; Kim, M.; Lee, S.-H.; Kim, Y.Y.; et al. Bioactive quinone derivatives from the marine brown alga *Sargassum thunbergii* induce anti-adipogenic and pro-osteoblastogenic activities. *J. Sci. Food Agric.* **2015**, *96*, 783–790. [\[CrossRef\]](#) [\[PubMed\]](#)
44. Pan, Y.; Wernberg, T.; de Bettignies, T.; Holmer, M.; Li, K.; Wu, J.; Lin, F.; Yu, Y.; Xu, J.; Zhou, C.; et al. Screening of seaweeds in the East China Sea as potential bio-monitors of heavy metals. *Environ. Sci. Pollut. Res.* **2018**, *25*, 16640–16651. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Yu, Y.Q.; Zhang, Q.S.; Tang, Y.Z.; Zhang, S.B.; Lu, Z.C.; Chu, S.H.; Tang, X.X. Establishment of intertidal seaweed beds of *Sargassum thunbergii* through habitat creation and germling seeding. *Ecol. Eng.* **2012**, *44*, 10–17. [\[CrossRef\]](#)
46. Li, X.M.; Zhang, Q.S.; Tang, Y.Z.; Yu, Y.Q.; Liu, H.L.; Li, L.X. Highly efficient photoprotective responses to high light stress in *Sargassum thunbergii* germlings, a representative brown macroalga of intertidal zone. *J. Sea Res.* **2014**, *85*, 491–498. [\[CrossRef\]](#)
47. Liang, Z.; Wang, F.; Sun, X.; Wang, W.; Liu, F. Reproductive biology of *Sargassum thunbergii* (Fucales, Phaeophyceae). *Am. J. Plant. Sci.* **2014**, *5*, 2574–2581. [\[CrossRef\]](#)
48. Stiger, V.; Horiguchi, T.; Yoshida, T.; Coleman, A.W.; Masuda, M. Phylogenetic relationships within the genus *Sargassum* (Fucales, Phaeophyceae), inferred from ITS-2 nrDNA, with an emphasis on the taxonomic subdivision of the genus. *Phycol. Res.* **2003**, *51*, 1–10. [\[CrossRef\]](#)
49. Zhao, F.; Wang, X.; Liu, J.; Duan, D. Population genetic structure of *Sargassum thunbergii* (Fucales, Phaeophyta) detected by RAPD and ISSR markers. *J. Appl. Phycol.* **2007**, *19*, 409–416. [\[CrossRef\]](#)
50. Li, J.-J.; Hu, Z.-M.; Gao, X.; Sun, Z.-M.; Choi, H.-G.; Duan, D.-L.; Endo, H. Oceanic currents drove population genetic connectivity of the brown alga *Sargassum thunbergii* in the north-west Pacific. *J. Biogeogr.* **2017**, *44*, 230–242. [\[CrossRef\]](#)
51. Gong, Y.; Suh, Y.-S.; Seong, K.-T.; Han, I.-S. *Climate Change and Marine Ecosystem*; Academy Book: Seoul, Korea, 2010; pp. 39–64.
52. Carruthers, T.J.B.; Longstaff, B.J.; Dennison, W.C.; Abal, E.G.; Aioi, K. Measurement of Light Penetration in Relation to Seagrass. In *Global Seagrass Research Methods*; Short, F.T., Coles, R.G., Short, C.A., Eds.; Elsevier Science: Amsterdam, The Netherlands, 2001; pp. 369–392.
53. Clarke, K.R.; Gorley, R.N. *PRIMER Version 6: User Manual/Tutorial*; Primer-E Ltd.: Plymouth, UK, 2006.
54. Umanzor, S.; Ladah, L.; Calderon-Aguilera, L.E.; Zertuche-González, J.A. Testing the relative importance of intertidal seaweeds as ecosystem engineers across tidal heights. *J. Exp. Mar. Biol. Ecol.* **2019**, *511*, 100–107. [\[CrossRef\]](#)
55. Anderson, M.J.; Gorley, R.N.; Clarke, K.R. *PERMANOVA+ for PRIMER: Guide to Software and Statistical Methods*; Primer-E Ltd.: Plymouth, UK, 2008.
56. Taylor, R.B.; Sotka, E.; Hay, M.E. Tissue-specific induction of herbivore resistance: Seaweed response to amphipod grazing. *Oecologia* **2002**, *132*, 68–76. [\[CrossRef\]](#)
57. Diaz-Pulido, G.; Villamil, L.; Almanza, V. Herbivory effects on the morphology of the brown alga *Padina boergesenii* (Phaeophyta). *Phycologia* **2007**, *46*, 131–136. [\[CrossRef\]](#)
58. Mueller, R.; Fischer, A.M.; Bolch, C.J.; Wright, J.T. Environmental correlates of phenotypic variation: Do variable tidal regimes influence morphology in intertidal seaweeds? *J. Phycol.* **2015**, *51*, 859–871. [\[CrossRef\]](#) [\[PubMed\]](#)
59. Pearson, G.A.; Davison, I.R. Freezing rate and duration determine the physiological response of intertidal fucoids to freezing. *Mar. Biol.* **1993**, *115*, 353–362. [\[CrossRef\]](#)
60. Dudgeon, S.R.; Kübler, J.E.; Vadas, R.L.; Davison, I.R. Physiological responses to environmental variation in intertidal red algae: Does thallus morphology matter? *Mar. Ecol. Prog. Ser.* **1995**, *117*, 193–206. [\[CrossRef\]](#)
61. Liu, F.; Pang, S.J. Stress tolerance and antioxidant enzymatic activities in the metabolisms of the reactive oxygen species in two intertidal red algae *Grateloupia turuturu* and *Palmaria palmata*. *J. Exp. Mar. Biol. Ecol.* **2010**, *382*, 82–87. [\[CrossRef\]](#)

62. Norton, T.A.; Mathieson, A.C.; Neushul, M. Morphology and Environment. In *The Biology of Seaweeds*; Lobban, C.S., Wynne, M.J., Eds.; University of California Press: Oakland, CA, USA, 1981; pp. 421–451.
63. Sjøtun, K.; Fredriksen, S.; Rueness, J. Effect of canopy biomass and wave exposure on growth in *Laminaria hyperborea* (Laminariaceae: Phaeophyta). *Eur. J. Phycol.* **1998**, *33*, 337–343. [[CrossRef](#)]
64. De Paula, E.J.; de Oliveira F<sup>o</sup>, E.C. Wave exposure and ecotypical differentiation in *Sargassum cymosum* (Phaeophyta-Fucales). *Phycologia* **1982**, *21*, 145–153. [[CrossRef](#)]
65. Viejo, R.M.; Arrontes, J.; Andrew, N.L. An experimental evaluation of the effect of wave action on the distribution of *Sargassum muticum* in northern Spain. *Bot. Marina* **1995**, *38*, 437–442. [[CrossRef](#)]
66. Chen, J.; Zhang, J.H.; Li, J.Q.; Zhang, Y.T.; Sui, H.D.; Wu, W.G.; Niu, Y.L.; Gao, Z.K. The growth characteristics of long-line cultured seaweed *Sargassum thunbergii* in the Sanggou Bay. *Prog. Fish Sci.* **2016**, *37*, 120–126.
67. Sideman, E.J.; Mathieson, A.C. Morphological variation within and between natural populations of non-tide pool *Fucus distichus* (Phaeophyta) in New England. *J. Phycol.* **1985**, *21*, 250–257. [[CrossRef](#)]
68. Stengel, D.B.; Dring, M.J. Morphology and in situ growth rates of plants of *Ascophyllum nodosum* (Phaeophyta) from different shore levels and responses of plants to vertical transplantation. *Eur. J. Phycol.* **1997**, *35*, 193–202. [[CrossRef](#)]