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Stipe Length as an Indicator of Reproductive Maturity in the Kelp *Ecklonia cava*

Sangil Kim[1], Suk Hyun Youn[1], Hyun-Ju Oh[1], Sun Kyeong Choi[2], Yun Hee Kang[3], Tae-Hoon Kim[3], Hyuk Je Lee[4], Kwang-Sik Choi[5], and Sang Rul Park[2]*

[1]*Ocean Climate & Ecology Research Division, National Institute of Fisheries Science, Busan 46083, Korea*
[2]*Estuarine & Coastal Ecology Laboratory, Department of Marine Life Sciences, Jeju National University, Jeju 63243, Korea*
[3]*Department of Earth and Marine Sciences, College of Ocean Sciences, Jeju National University, Jeju 63243, Korea*
[4]*Department of Biological Science, Sangji University, Wonju 26339, Korea*
[5]*School of Marine Biomedical Science (BK21 PLUS), Jeju National University, Jeju 63243, Korea*

Abstract – We conducted testing to determine whether stipe length is a useful indicator of reproductive maturity in the kelp *Ecklonia cava*, a species that plays a pivotal role in ecosystem functioning and services in subtidal areas. Approximately 100 sporophytes with stipes of various lengths were collected during the fertile period (July–November, 2013). We investigated the relationships between stipe length and other morphological characteristics to determine whether stipe length could indicate the age of reproductive maturity. Primary blade length, longest blade length, thallus height, and total length showed significant relationships with stipe length. The length of the primary and longest blades gradually declined as stipe length increased above 125 mm. Zoosporangial sori were found on the blades of more than 70% of individuals with stipes longer than 125 mm, but on only 8% of individuals with stipes less than 125 mm long. Stipe length therefore seems to be an acceptable proxy for reproductive maturity. Another factor to consider, however, is that all specimens with zoosporangial sori, regardless of stipe length, had a dry weight of 80 g or more; thus, individual biomass may also be an important parameter influencing the initiation of reproduction.

Keywords – *Ecklonia cava*, individual biomass, morphology, reproductive maturity, stipe length

1. Introduction

Kelp forests are a central component of coastal marine ecosystems, dominating shallow rocky subtidal zones in the world's temperate and polar coastal oceans (Steneck et al. 2002). Kelps are a vital component of detritus-based food webs in these ecosystems, due to low-pressure herbivory (Duggins et al. 1989; Mann 2000). Kelp forests provide habitats, shelter, and nursery areas for numerous sessile animals and algae as well as mobile pelagic and benthic organisms (Reed and Foster 1984; Dunton and Schell 1987; Levin 1994; Anderson et al. 1997). They also reduce water flow and coastal erosion, and stabilize sediment (Duggins et al. 1990). Recent attention has focused on the carbon sequestration potential of kelp forests, which capture carbon via algal photosynthesis (Nellemann et al. 2009). Despite the ecological importance of kelp forests, in many parts of the world these ecosystems are declining, as they are being threatened by anthropogenic impacts including overexploitation, enriched nutrient availability, and climate change (Steneck et al. 2002; Krumhansl et al. 2016). To address this issue, several countries have established kelp forest management policies or preserves under various laws (e.g., Kim et al. 2017).

Kelp forests in temperate bioregions are characterized by their dominant genera (e.g., *Laminaria*, *Ecklonia*, and *Macrocystis*) (Mann 2000). Kelp forests in New Zealand, southern Australia, South Africa, and the northwestern Pacific are dominated by *Ecklonia* species (Mann 2000; Steneck et al. 2002). On the eastern and southern coasts of Korea, including the area around Jeju Island, the dominant species is *Ecklonia cava* Kjellman, which grows in shallow sublittoral zones (Kang 1966; Kim et al. 2017). A dense canopy-forming algae, this species acts as an "ecosystem engineer," modifying the

*Corresponding author. E-mail: srpark@jejunu.ac.kr

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surrounding abiotic environment and impacting other organisms (Jones et al. 1994). *Ecklonia cava* is a primary producer and also provides important habitats, including nursery areas, for commercially and ecologically valuable marine organisms in subtidal rocky reefs (Ohno 1985; Yokohama et al. 1987; Kim 2016). Recently, this species has been increasingly cultivated as a food for abalone and serves as a significant carbon dioxide sink due to its low production costs and high productivity (Chung et al. 2013; Hwang et al. 2013). It is also widely used as a raw material for functional foods, dietary supplements, and pharmaceuticals (reviewed by Koirala et al. 2017), and to artificially restore large seaweed forests in barren areas (Terawaki et al. 2001; Kim et al. 2013a, 2013b).

Understanding the population ecology of kelps is the first step in creating management policies for conservation or restoration of kelp-based ecosystems. Kelp biomass and extent can only be predicted if there is adequate background information on the biological and environmental factors that affect the increase, decline, or maintenance of kelp populations. In particular, reproduction and recruitment, which are affected by potential fecundity, contribute to population expansion and are critical processes in population dynamics. Therefore, assessing the potential fecundity of kelps—by determining their reproductive age, the sorus (reproductive organ) area per individual and the number of total spores per sorus—is a necessary prerequisite to accurate prediction of future population dynamics, which is a crucial component in determining kelp conservation policy.

Several studies have shown that stipe lengths, in relation to other morphometric parameters, can be used as an index of age in several kelp species (Klinger and DeWreede 1988; Pedersen et al. 2012). The formation or maturity of reproductive structures is probably related to plant age or size (Chapman 1986; Ang 1991; Coleman et al. 1994). Therefore, stipe length, as an indicator of age, may also be a critical indicator of the formation or maturity of reproductive structures in some kelp species. The objective of this study was to use morphological characteristics to predict the age of reproductive maturity in *E. cava* before the formation of reproductive structures (so-called 'zoosporangial sori'). To this end, we examined the relationships between morphological characteristics, stipe length, and the ratio of individuals with zoosporangial sori to evaluate the age of reproductive maturity. Our results will provide invaluable information in creating management policy for the restoration of *E. cava* ecosystems.

2. Materials and Methods

This study was conducted in subtidal areas at Eoyeong (northern coast) and Munseom (southern coast) on Jeju Island in Korea's southern sea (Fig. 1). There is a remarkable difference in seawater temperature between the north and
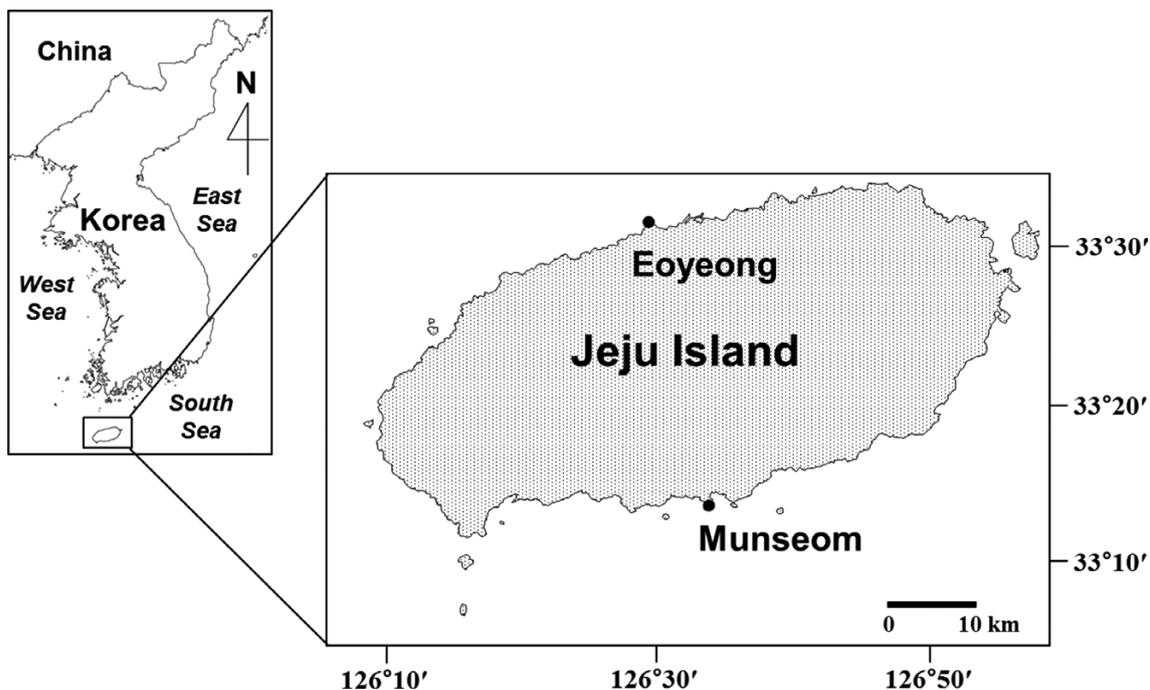

Fig. 1. Study areas at Eoyeong (northern coast) and Munseom (southern coast) on Jeju Island, Korea

south sides of Jeju Island; the average winter water temperature at Eoyeong (14°C) is lower than at Munseom (16°C, Kim 2016). Salinity was 29.8–34.3 PSU throughout the experimental period at both sites (Kim 2016). The tidal regime of this area is semi-diurnal and the system is classified as mesotidal, with tidal amplitude ranging from about 0.7 m during neap tides to 2.7 m during spring tides (Tide Tables for the Coast of Korea, National Oceanographic Research Institute of Korea). Macroalgal assemblages in the subtidal zone around both sites are dominated primarily by geniculate and crustose coralline algae (*Lithophyllum okamurae* and *Amphiroa* spp.) and large brown algae (*E. cava* and *Sargassum* spp.). Especially, *E. cava* forms dense assemblages between 8 and 12 m of water depth.

We collected 100 sporophytes with stipes of various sizes

from largely monospecific *E. cava* forests between 8 and 10 m water depth at Eoyeong and Munseom, Jeju Island during July–November 2013 when this species forms reproductive structures. The collected samples were transported to the laboratory using paper moistened with seawater to prevent water loss, and processed immediately. Stipe length, lengths of the primary and longest blades, thallus height, and total length (stipe length + longest blade length) of each sporophyte were measured to the nearest 1.0 mm (see illustrations in Kim et al. 2017). The blades of all samples were examined for zoosporangial sori.

A regression analysis was performed to investigate the relationships between stipe length and other morphological characteristics (lengths of the primary and longest blades, thallus height, and total length). The best fit for relationships
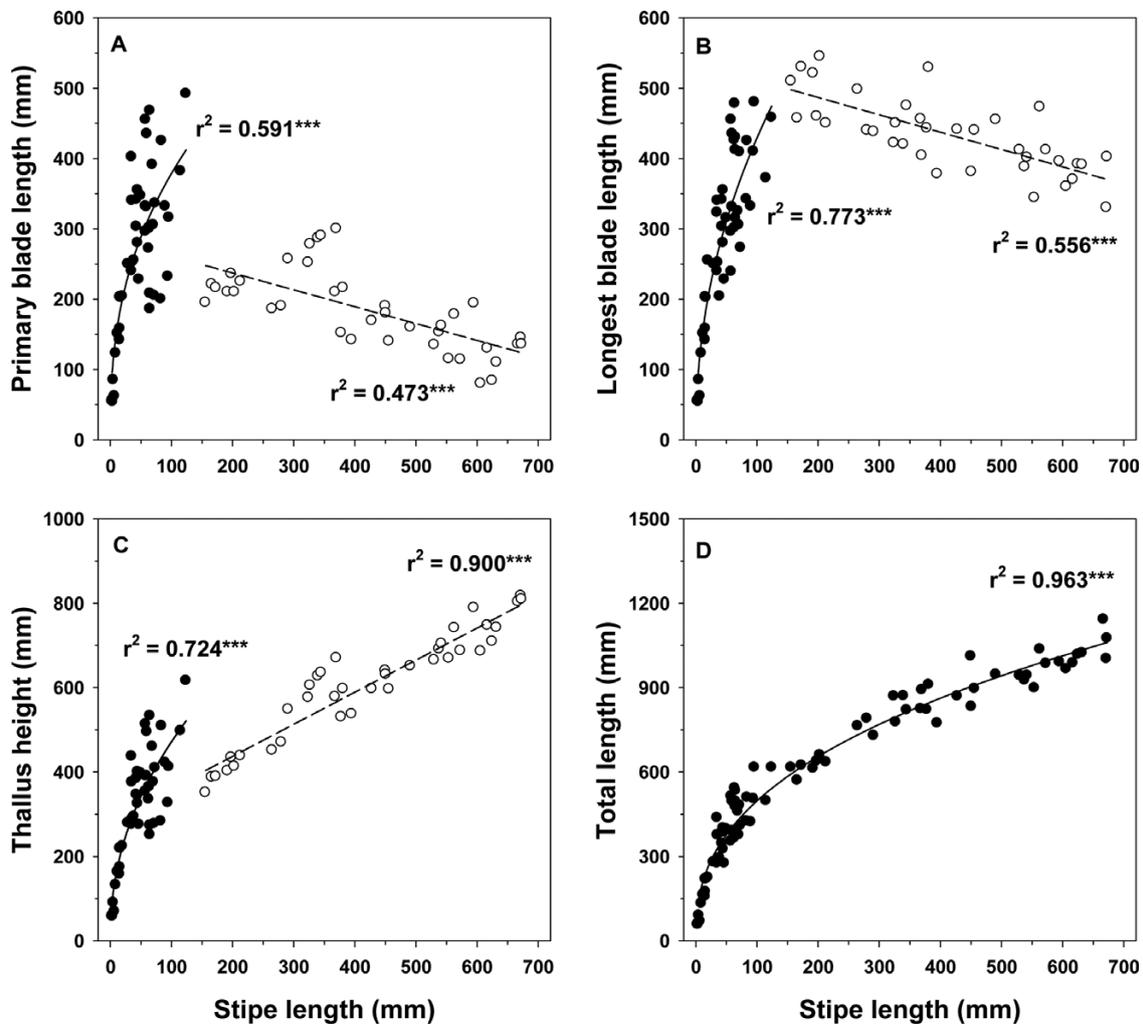

Fig. 2. Relationships between stipe length and morphological characters (A, primary blade length; B, longest blade length; C, thallus height, and D, total length) in *Ecklonia cava*. Relationships between stipe length and morphological characteristics were fitted using the equations Y = aX^b (solid lines) or Y = aX + b (dashed lines). $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$

Table 1. Relationships between stipe length (X) and morphological characters (Y; primary blade length, longest blade length, thallus height, and total length)

Stipe length (mm)	Morphological character (mm)	Regression model	a	b	r²
$0 < X \leq 125$	Primary blade length	$y = ax^b$	61.832	0.394	0.591
	Longest lateral blade length		50.260	0.466	0.773
	Thallus height		55.674	0.464	0.724
$125 < X$	Primary blade length	$y = ax + b$	-0.240	284.895	0.473
	Longest lateral blade length		-0.247	535.893	0.556
	Thallus height		0.760	284.895	0.900
All data	Total length	$y = ax^b$	79.771	0.397	0.963

between stipe length and morphological characteristics were found to be power or polynomial models. All regression analyses were conducted using SPSS software (version 18.0; SPSS Inc., USA).

3. Results

Stipe length exhibited a significant relationship with morphological characteristics (primary blade length, longest blade length, thallus height, and total length; Fig. 2, Table 1). Indeed, stipe length explained more than 90% of the variation ($r^2 = 0.963$) in total length (Fig. 2D). However, the relationships between primary blade length, longest blade length, thallus height, and stipe length fell into two distinct phases (Fig. 2A–C). In the first phase, seen in plants with stipes less than 125 mm long, primary blade length, longest blade length, and thallus height dramatically increased with stipe length (Fig. 2A–C, Table 1). The second phase began when stipe length reached 125 mm; after this point, primary blade length and longest blade length decreased linearly with increasing stipe length, while thallus height continued to increase in a linear manner (Fig. 2A–C, Table 1).

The density of zoosporangial sori on the blades of sporophytes was higher in specimens with stipe lengths of 125 mm or more (Table 2). Only 8% of individuals with stipes less than 125 mm long had zoosporangial sori on their blades, whereas zoosporangial sori were observed on the blades of more than 70% of individuals with stipes of 125 mm or longer (Table 2).

Table 2. Stipe length (≤ 125 mm or >125 mm) and ratio of individuals with zoosporangial sori at Eoyeong and Munseom on Jeju Island, Korea

Stipe length (mm)	Individuals with zoosporangial sori (%)
≤ 125	8.0
> 125	71.0

4. Discussion

The initiation of reproduction is related to age in several kelp species (DeWreede and Klinger 1988; Notoya and Aruga 1990). In *E. stolonifera* specimens of well-determined age, the formation of zoosporangial sori was observed in 3-year-old sporophytes (Park et al. 1994). However, for practical purposes, it is impossible to predict reproductive maturity using sporophyte age due to the lack of age-specific data, and because the relationship between age and the prevalence of sexually mature sporophytes is unknown (Graham et al. 1997). Thus, morphological characteristics, such the diameter and length of stipe and blade length, rather than age, are generally used to predict reproductive maturity (Park et al. 1994; Mabin et al. 2013). In both the short-stiped *Saccharina latissima* and the long-stiped *S. longicruris*, zoosporangial sori were observed only on individuals with blades longer than 750 mm (Krause-Jensen et al. 2012). Graham et al. (1997) found that zoosporangial sori first appear in adult sporophytes of *Macrocystis pyrifera*, i.e., those with holdfast diameters of 140 mm or more (Graham et al. 1997). In the present study, remarkable morphological changes occurred in sporophytes when stipe length passed 125 mm. The lengths of the primary and longest blades and thallus height increased until the stipe reached a length of 125 mm; thereafter, these measurements gradually decreased with increasing stipe length. In contrast, thallus height increased with stipe length after 125 mm. Surprisingly, the frequency of zoosporangial sori dramatically increased in sporophytes with stipe lengths of over 125 mm. This indicates that reproductive maturity (formation of zoosporangial sori) occurs largely in sporophytes with stipes of 125 mm or longer. Therefore, stipe length is the most practical morphological measure to use as an indicator of the initiation of reproduction in *E. cava*.

When zoosporangial sori were observed on the blades of

S. longicruris, stipe length was more than 150 mm; however, a few individuals with stipes less than 100 mm did have zoosporangial sori on their blades (Chapman 1973). In this study, we observed zoosporangial sori on the blades of a few sporophytes with stipes shorter than 125 mm. The average individual biomass of these sporophytes was 80 g dry weight. Interestingly, the individual biomass of sporophytes with stipe lengths greater than 125 mm that did not have zoosporangial sori was less than 75 g dry weight. Lees (2001) reported that *E. radiata* in Wainui and Ohinepaka Bays developed zoosporangial sori at a minimum of 122–160 g fresh weight. The maturity of *Laminaria hyperborea* was related mainly to frond weight, occurring between approximately 30 and 80 g fresh weight in winter (Kain 1975). This indicates that the formation of zoosporangial sori is related to individual biomass; sporophytes must achieve adequate individual biomass to develop zoosporangial sori.

After recruitment, how many years does it take for the stipe length of *E. cava* sporophytes to reach 125 mm, and thus for the plant to attain reproductive maturity? Serisawa et al. (2003) reported that the stipe lengths of 1–2-year-old *E. cava* specimens were 80–240 mm, with an average of 140 mm. Stipe lengths of *E. cava* in rope culture reached 130 mm, on average, 14 months after artificial settlement (Hwang et al. 2013). *Ecklonia radiata* in Wainui and Ohinepaka Bays developed zoosporangial sori at a minimum age of 1.57–1.68 years (Lees 2001). Recruits in the coastal marine areas of Jeju Island reach adulthood within 1.0–1.33 years, at which time they have an average stipe length of 125 mm (S Kim, unpublished data). Thus, *E. cava* likely achieves complete reproductive maturity 12–16 months after recruitment.

In conclusion, we uncovered a significant change in the morphological characteristics of *E. cava* once stipe length reached 125 mm. After this point, the lengths of the primary and longest blades gradually decreased. Zoosporangial sori were found on the blades of most sporophytes of this size, whereas only a few individuals with stipes less than 125 mm in length developed zoosporangial sori. This relationship between stipe length and the frequency of zoosporangial sori suggests that stipe length is a useful characteristic in determining when an *E. cava* specimen reaches reproductive maturity. Additionally, considering the relationship between individual biomass and the presence of zoosporangial sori, mass may be another useful indicator for evaluating the age of reproductive maturity of *E. cava*.

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