

Use of morphological characteristics for calculating individual biomass in the kelp *Ecklonia cava*

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Abstract Because kelp biomass contributes to the abundance and production of myriad animals and plants in coastal marine ecosystems, its measurement is critical to evaluating the impact of macroalgae on marine and aquatic habitats. We investigated the relationships among morphological characteristics to develop a non-destructive method for calculating *Ecklonia cava* biomass. Approximately 253 sporophytes of *E. cava* with various stipe size gradients were collected at five sites on a seasonal basis from November 2012 to August 2014. The relationships between morphological characteristics and individual weight were best fit by a non-linear regression equation, the sigmoidal regression equation. The length and diameter of the stipe, blade number, and total length accounted for more than 80% of the variation in individual weight. In particular, total length × blade number was highly correlated ($r^2 = 0.94$) correlated with individual weight. The non-destructive method was 3.4–6.7 times faster than destructive sampling method. Considering the cost and time of fieldwork, stipe diameter and length are suitable for large-scale long-term monitoring. We recommend the use of the total length × blade number variable to improve the accuracy of plant biomass estimation in monitoring programs.

Keywords *Ecklonia cava* · Phaeophyceae · Kelp biomass · Morphometric equation · Non-destructive method

Introduction

Kelp forests are identified as an important component of coastal marine ecosystems and provide habitat, shelter, and nursery areas for marine organisms (Steneck et al. 2002). They influence water flow, coastal erosion, and sedimentation by dampening wave action (Duggins et al. 1990). Recently, kelp forest ecosystems have received considerable attention as a carbon sequestrator (Nellemann et al. 2009). However, they are declining significantly in many parts of the world due to biotic and abiotic stresses (disease, herbivory, fishing, climate change, nutrient availability, and storms) and interactions among those processes (reviewed by Steneck et al. 2002). Thus, kelp forests are preserved or protected by various laws in Australia (Environment Protection and Biodiversity Conservation Act 1999), the European Union (the law on Management of Wild Marine Resources), Japan (Acts of the Protection of the Fisheries Resources), and the USA (Kelp and Marine Aquatic Plant Laws).

Kelp forests are among the most productive ecosystems on the planet (Steneck et al. 2002). Because kelp biomass contributes to the abundance and production of myriad animals and plants in coastal marine ecosystems, its measurement is critical for evaluating the impact of macroalgae and the structure and function on marine and aquatic habitats (Reed et al. 2008). Kelp biomass can be used as a proxy of habitat productivity and can provide a means of assessing carbon sequestration, both biological and economic (Reed et al. 2008; Laffoley and Grimsditch 2009; Chung et al. 2013). Traditionally, kelp is removed from the field using 1 m² quadrats, transported to the

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laboratory and then weighed to measure biomass (the “destructive sampling method”). However, this method is labor-, cost-, and time-intensive as biomass sampling requires SCUBA diving for long periods. In addition, this method limits the area of kelp bed that can be sampled or monitored and is destructive to plant.

A number of non-destructive methods have been proposed to complement the destructive sampling method. A non-destructive assessment method using the equation derived from the relationship between percent cover and biomass can be applied to various macroalgae (Rollon et al. 2003) but can yield over- or underestimates due to the inaccuracy of percentage estimates obtained based on the three-dimensional morphology of kelp (Robbins and Boese 2002). The usefulness of remote-sensing techniques for estimating kelp biomass has been investigated (North et al. 1993; Stekoll et al. 2006). Although remote-sensing techniques have been used successfully to map kelp beds, these techniques estimate kelp biomass using data obtained from destructive sampling (Stekoll et al. 2006; Anderson et al. 2007).

The allometric method for estimating kelp biomass using biomass-morphological characteristics was developed to overcome the inherent disadvantages of removing material from the field and to improve the accuracy of estimates (Rothman et al. 2010). The high correlation between kelp biomass and morphological characteristics indicated the usefulness of this method. This method has been evaluated as a simple and easy assessment technique for estimating kelp biomass. In contrast, the regression equation for the relationship between kelp biomass and morphological characteristics may strongly vary with locations. Additionally, long-term monitoring of kelp biomass is essential to capture seasonal and yearly variations. Thus, sampling for allometric equation to estimate biomass should consider spatial and temporal variations.

The kelp *Ecklonia* is the dominant genus of kelp forests in South Australia, Northeast Asia, and South Africa (Mann 2000). In particular, *Ecklonia cava* Kjellman, a species native to North Pacific waters off the coasts of Korea and Japan, is the major habitat-forming organism in subtidal areas along the southern coast of Korea (Bolton and Anderson 1994). *Ecklonia cava* plays an important role as a food source in kelp-dominated ecosystems (Kim 2016) and is the major food for abalone due to its huge biomass (Hwang et al. 2013). In addition, it is a major source of bioactive components (Artan et al. 2008; Thomas and Kim 2011). Thus, the Korean government prohibits collection of this species for commercial use under the Fishery Resource Management Act (Law no. 10599). In this study, we identified a relationship that would facilitate calculation of the biomass or productivity of *E. cava* from a simple, easy and costless, non-destructive measure for use in long-term monitoring. To this end, we performed annual sampling of *E. cava* with stipes of various sizes from five

sites seasonally during the entire experimental period (2012–2014).

Materials and methods

Study area

Jeju Island, located in the southern sea of Korea, is the largest island in Korea. The island coastline stretches along approximately 308 km and comprises 75% natural rocky shores, 17.9% artificial hard-bottom shores, and 7.1% sand beaches (<http://www.Khoa.go.kr>). Because the Tsushima Current (a branch of the Kuroshio Current) flows around Jeju Island, the climate of Jeju Island is strongly affected by the warm Kuroshio Currents. The water temperature was 13.7–26.6 °C during the last 17 years (1992–2008; <http://www.Khoa.go.kr>). Average water temperature during winter was 2 °C higher on southern coast of Jeju Island than on northern coast of Jeju Island (Kim 2016). Strong typhoons are frequent during summer–early autumn. Annual mean precipitation from 1971 to 2000 was approximately 1975 mm, about 60% of which occurred during the summer monsoon season (<http://www.kma.go.kr>). The tidal regime is semi-diurnal with a maximum tidal range of about 2.7 m during spring tides (Tide Tables for the Coast of Korea, National Oceanographic Research Institute of Korea). The benthic community structure in the subtidal zone is dependent on water depth (Kim et al. 2013). *Lithophyllum okamurae*, *Amphiroa anceps*, *Amphiroa ephedraea*, *Amphiroa misakiensis*, and *Codium coactum* dominate at 0–5 m water depth. Large brown algae such as *Ecklonia cava* and *Sargassum* spp. are abundant at a depth of 5–15 m. In particular, *E. cava* forms dense assemblages at depths of 8–12 m. The predominant species at 15–30 m depth are the red alga *Peyssonnelia capensis* and corals such as *Scleronephthya gracillimum*, *Dendronephthya* spp., and *Alveophora japonica*.

Data collection

A total of 253 plants were collected at five sites along the coast of Jeju Island from November 2012 to August 2014 (Table 1). Individuals were selected to cover the entire range of size variation within populations, including recruits, juveniles, and adults (Table 2). All samples were taken at 8–12 m depth, from a dense, near-homogeneous stand of *E. cava*, transported to the laboratory using paper moistened with seawater to prevent water loss from samples, and processed immediately. The diameter and length of the stipe, lengths of the primary and longest blade, thallus height, and total length (stipe length + longest blade length) of each plant were measured (Fig. 1). The number of blades per

Table 1 Study sites, their position (latitude, longitude, and location), collection dates, and sampling size on Jeju Island, Korea

Site	Latitude (N)	Longitude (E)	Location	collection date	Sample size (<i>n</i>)
Munseom	33° 13' 38"	126° 34' 04"	Southern coast	November 2012; March, July, September, and November 2013; May and August 2014	105
Pyoseon	33° 18' 26"	126° 48' 56"	Southeastern coast	September 2013; February and June 2014	21
Seongsan	33° 27' 42"	126° 56' 24"	Eastern coast	September 2013; March and July 2014	30
Dodoo	33° 24' 12"	126° 13' 32"	Northwestern coast	September 2013; April and August 2014	30
Eoyeong	33° 31' 11"	126° 29' 20"	Northern coast	November 2012, March, June, and September 2013; April and July 2014	67

plant was counted. Then, all samples were thoroughly cleaned of epiphytes and sediment, separated into stipe and blade parts, and dried at 60 °C to constant weight.

Statistical analyses

All thalli ($n = 253$) were used for evaluation of the allometric relationships between morphological characteristics and individual weight. The relationships between morphological characteristics and individual weight were best fit by various non-linear regression equations (sigmoidal, exponential rise to maximum, logistic, hyperbola, and power law equations). Then, we selected for explanatory variables the sigmoidal model using the Akaike information criterion (AIC) and the determination coefficient (r^2). The explanatory variables include one variable (stipe diameter, stipe length, primary blade length, longest blade length, blade number, thallus height, total length) or combination of variables (variable \times variable). All statistical analyses were performed using R software (Version 2.9.0).

Results

Morphology of *E. cava*

Ecklonia cava collected in this study exhibited highly variable morphological characteristics (Table 2). The stipe length of *E. cava* collected ranged from 3.0 to 894.0 mm. Regarding stipe diameter, the median value (11.78 mm) was slightly higher than the mean value (10.38 mm). Primary blade length ranged from 54.0 to 492.0 mm, while longest lateral blade length ranged from 27.0 to 581.0 mm (Table 2). Thallus height varied from 5.8 to 105.1 cm during the experimental period (Table 2). Individual weights varied from 0.03 to 160.52 g dry weight (Table 2). The stipe/blade biomass ratios increased gradually with increasing stipe length (Table 2).

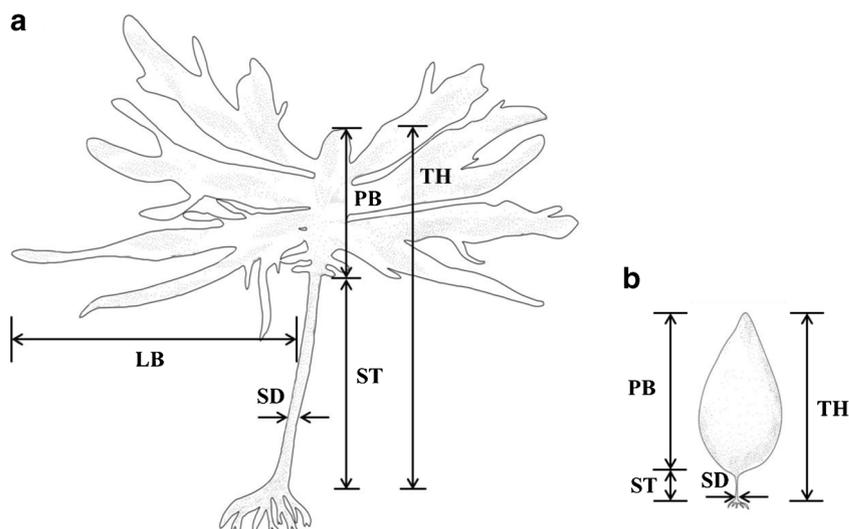
Morphological characteristics vs. individual weight

The relationships between morphological characteristics and individual weight in *E. cava* were established to find a simple

Table 2 Summary of stipe class and diameter, blade length and number, thallus height, individual weight, and contribution of stipe biomass to the total biomass (S/T ratio) of kelp collected on Jeju Island, Korea ($n = 253$)

Stipe class (mm)	Frequency (%)	Stipe diameter (mm)	Blade length (mm)		Thallus height (cm)	Blade number	Individual weight (g dry weight)	S/T ratio
			Primary	Longest lateral				
1–20	9.3	1.0–2.4	54–203	–	5.8–21.9	1	0.028–0.472	0.04
21–50	10.2	2.9–6.2	204–402	27–323	24.3–43.7	6–17	0.510–14.175	0.07
51–70	11.0	4.9–9.6	186–468	94–478	25.1–53.3	10–37	2.670–78.439	0.04
71–100	6.8	4.2–10.0	200–425	55–520	27.7–50.9	10–41	1.421–109.526	0.07
101–200	11.9	8.0–12.3	118–492	290–477	28.0–61.6	20–33	19.801–80.916	0.07
201–300	10.2	9.8–13.4	115–257	319–515	37.8–54.8	22–34	35.165–94.235	0.11
301–400	10.2	11.9–16.0	142–322	277–581	53.0–70.1	21–43	38.123–132.110	0.14
401–500	8.5	13.8–15.0	140–370	381–561	59.6–85.7	25–46	56.028–117.238	0.17
501–600	7.6	12.7–19.0	80–213	344–526	70.9–78.9	18–47	42.129–154.489	0.22
601–700	6.8	13.8–18.4	84–240	282–475	68.6–92.4	26–55	75.496–160.522	0.21
701–800	5.1	13.8–17.4	106–155	316–406	83.3–91.2	22–39	72.062–140.823	0.25
800–900	1.8	19.3–21.07	140–195	425–436	103.4–105.1	37–43	137.560–142.071	0.25

Fig. 1 Biometric parameters used to evaluate the *Ecklonia cava* adult (a) and juvenile (b) thallus. *ST* stipe length, *SD* stipe diameter, *PB* primary blade, *LB* lateral blade, *TH* thallus height



and easy allometric equation. In this study, we tested 7 explanatory variables (one variable) and 20 combination variables (variable \times variable) except thallus height \times total length variable. The 12 selected morphological characteristics–biomass relationships are shown in Fig. 2, and their respective parameters are given in Table 3. Significant relationships were found between all morphological characteristics, with the exception of primary blade length and individual weight in *E. cava* (Fig. 2). Total length exhibited a significant relationship with individual weight and explained 85% of the variation in individual weight (Fig. 2f). More than 80% of the variation in individual weight was explained by stipe diameter, stipe length, and blade number (Fig. 2a, b, d). Longest blade length and thallus height accounted for 61 and 67% of the observed variation in individual weight, respectively (Fig. 2c, e). Six variables across 20 combination variables explained more than 80% of the variation in individual weight (Fig. 2g–l). In particular, total length \times blade number was highly ($r^2 = 0.94$) correlated with individual weight, and accounted for more than 90% of the variation in that parameter (Fig. 2l).

Discussion

The method of determining kelp biomass from destructive sampling is widely used but requires prolonged SCUBA diving, and retrieving the whole thallus in the field is time- and cost-intensive (Rothman et al. 2010). For example, destructive sampling method took approximately 0.67 diver-hours to collect samples within a 1-m² quadrat in this study. Therefore, several studies have attempted to identify empirical allometric relations to enable estimation of kelp biomass in the field (Gendron 1985; Gevaert et al. 2008; Rothman et al. 2010). In this study, all morphological characteristics, with the exception of primary blade length, were significantly correlated with the

individual weight of *E. cava*, in that they explained more than 60% of the variation. Measuring one morphological character took 0.1–0.2 diver-hours per 1 m² quadrat in the field. Non-destructive method was 3.4–6.7 times faster than destructive sampling method. This suggests that use of morphometric characteristics is a cost-effective technique for evaluating kelp biomass (Gevaert et al. 2008; Rothman et al. 2010).

Of the 27 explanatory variables investigated, total length \times blade number was the best predictor of individual weight. This may be related to the characteristics of the total length \times blade number parameter, which comprises three parameters (stipe length, longest blade length, and blade number). In *E. maxima*, stipe length is useful for estimating plant biomass (Rothman et al. 2010). The whole-plant biomass of *Macrocystis* can be estimated from individual frond length (Stekoll and Else 1992; van Tamelen and Woodby 2001). Blade biomass has been used to calculate the whole-plant biomass of kelp species such as *Nereocystis luekeana* and *Alaria fistulosa* (Stekoll et al. 2006). In the present study, stipe length, longest blade length, and blade number showed significant relationships with individual weight. The blade biomass dependent on longest blade length and blade number accounted for 75–96% of whole plant biomass (Table 2). This is likely to increase the accuracy of estimation of plant weight using total length \times blade number. Despite its advantages, determination of the total length \times blade number parameter requires prolonged SCUBA diving (0.35 diver-hours per 1 m² quadrat). Therefore, this method is suitable for monitoring programs that require accurate estimation of the biomass in kelp forests.

Of one morphological character, total length was significantly correlated with individual weight ($r^2 = 0.85$). These results suggest that total length is an effective substitute for weighing the whole plant. Similar to total length, thallus height (stipe length + primary blade length) explained more

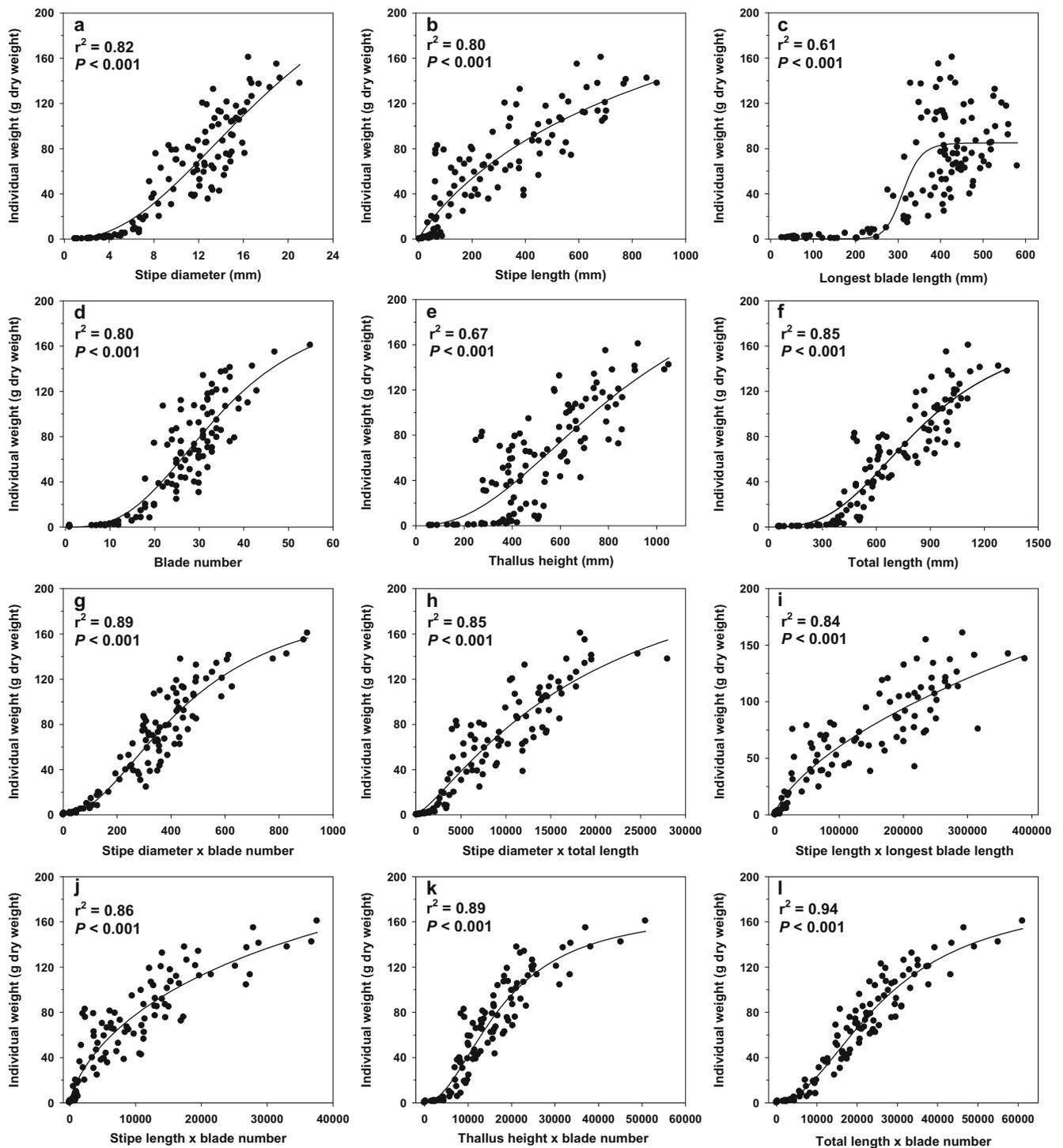


Fig. 2 Allometric relationships between individual weight and morphological characteristics (a stipe diameter, b stipe length, c longest blade length, d blade number, e thallus height, f total length, g stipe diameter × blade number, h stipe diameter × total length, i stipe length

× longest blade length, j stipe length × blade number, k thallus × blade number, l total length × blade number) in *Ecklonia cava*. Relationships between stipe length and morphological characteristics were fitted using the equations: $Y = a / [1 + (X - X_0)^b]$ ($n = 253$)

than 67% of the variation in individual weight. The ability to predict individual weight based on thallus height was slightly lower than that of total length, likely due to the variability of primary blade length in thallus height. The highest individual

weight has been observed at about 150–200 mm of primary blade length, after which individual weight decreased with increasing primary blade length (data not shown). The ratio of primary blade length to thallus height decreased with

Table 3 The relationships between morphological characteristics and individual weight in *Ecklonia cava* in Jeju Island, Korea

Variable (X)	a	X_0	b	r^2
Stipe diameter (mm)	288.31	19.81	-2.31	0.82
Stipe length (mm)	327.29	1244.86	-0.89	0.80
Longest blade length (mm)	85.03	312.36	-14.66	0.61
Blade number	201.21	34.81	-2.93	0.80
Thallus height (mm)	248.13	879.94	-2.23	0.67
Total length (mm)	178.71	854.48	-2.91	0.85
Stipe diameter \times blade number	196.04	458.32	-2.01	0.89
Stipe diameter \times total length	251.39	19,319.64	-1.27	0.85
Stipe length \times longest blade length	754.36	3,039,228.55	-0.72	0.84
Stipe length \times blade length	360.45	59,534.65	-0.72	0.86
Thallus height \times blade number	169.93	17,646.35	-1.99	0.89
Total length \times blade number	181.97	26,290.31	-1.99	0.94

Sigmoidal regression equation: Individual weight = $a / [1 + (X / X_0)^b]$

increasing stipe length (Table 2). These results indicate that although thallus height does not differ among plants, individual weight can vary with age or maturity level. Therefore, thallus height may not be a satisfactory predictor of individual weight in populations of plants with various ages.

The usefulness of stipe diameter for estimating plant biomass has been reported in several kelp species (Dean et al. 1996; Vásquez and Vega 2004; Stekoll et al. 2006). Although the use of stipe diameter as a predictor of age is limited by the variation in growth rate among individuals, stipe diameter shows a strong relationship with individual age, reflecting the degree of plant maturity (Klinger and DeWreede 1988). In the present study, the stipe diameter showed a strong correlation ($r^2 = 0.82$) with individual weight. In the field, stipe diameter can be measured more easily and rapidly (0.1 diver-hours per 1 m² quadrat) than other parameters. Therefore, this method is both less laborious and more cost-effective due to the reduced SCUBA diving time required. However, stipe diameter varies markedly according to location and season (Stekoll et al. 2006). The stipe diameter is greater at exposed than at sheltered areas (Bekkby et al. 2014). A tiny difference of stipe diameter may potentially cause a large difference between the predicted biomass and the observed biomass. Therefore, use of the stipe diameter to estimate individual weight should be approached with caution.

In conclusion, determining plant biomass using morphometric characteristics is suitable for non-destructive assessment of kelp species as it enables more simple and cost-effective assessment of kelp forest. However, the allometric equations should be considered spatial and temporal variations of individual weight in *E. cava*. Despite the drawbacks of the stipe diameter variable, the stipe diameter and length are still appropriate for long-term monitoring in kelp beds at a large scale due to the simplicity and effectiveness of the equation. Total length consisting of stipe length and longest blade length reflects stipe and blade weights, which enables

estimation of plant biomass at a medium scale in consideration of diving time and accuracy of this method. Total length \times blade number enables accurate assessment of individual weight as it takes into consideration stipe length, longest blade length, and blade number. We recommend the use of this parameter to predict *Ecklonia* plant biomass in monitoring programs.

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