Growth and reproductive patterns of *Undaria pinnatifida* sporophytes in a cultivation farm in Busan, Korea

Han Gil Choi · Young Sik Kim · Soon Jeong Lee · Ki Wan Nam

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Abstract Monthly growth and reproduction of *Undaria pinnatifida* sporophytes were examined over a period of 5 months in a cultivation farm in Korea. A total of 11 characters of *Undaria* were measured to determine a reliable morphological character representing its growth and reproduction. Plant weight of *Undaria* sporophytes increased steadily over the experimental period, but it increased in four different ways. *Undaria pinnatifida* increased body weight by growth in length and width (October–early December), and by growth in width with the thickening of blade and stipe when sporophytes began to be fertile (December–January). In the middle of January, growth in length and width had almost stopped with the maturation of *Undaria* sporophytes. Finally, the weight of *Undaria* increased again by growth in width at the

end of February. Present results indicate that *Undaria* sporophytes increase body weight by growth in length and width at different times, and the relationship between reproduction and vegetative growth is exclusive. Plant weight was positively correlated and fitted well with stipe width and blade width. The blade of *Undaria* was very thin (ca. 254 μm) and breakable by wave action, but its stipe was strong and relatively thick (ca. 8.7 mm). Furthermore, the fertility of *U. pinnatifida* was fitted better with stipe width than blade width. Thus, we suggest that the stipe width is the most feasible character with which to estimate the growth and reproduction of *U. pinnatifida* sporophytes in the cultivation farm.

Key words aquaculture · growth · reproduction · stipe width · sporophyte · *Undaria pinnatifida*

H. G. Choi (✉)
Faculty of Biological Science and Research Institute
for Basic Science, Wonkwang University,
Iksan, Jeonbuk 570-749, South Korea
e-mail: hgchoi@wonkwang.ac.kr

Y. S. Kim
School of Marine Life Science,
Kunsan National University,
Kusan, Jeonbuk 573-701, South Korea

S. J. Lee · K. W. Nam
Department of Marine Biology,
Pukyong National University,
Busan, 608-737, South Korea

Introduction

Undaria pinnatifida (Harvey) Suringar, a brown alga endemic to Korea, Japan and China, has extended its geographical distribution to the shores of Europe through introduction for aquaculture, and to New Zealand by maritime traffic (Yamanaka and Akiyama 1993; Castric-Fey et al. 1999a; Stuart et al. 1999). Moreover, *U. pinnatifida* is important as a nursery

ground for marine animals and as food for marine animals and humans in Asian countries (Koh and Shin 1990; Yamanaka and Akiyama 1993; Sohn 1998).

It is an annual species with a life cycle typical of the Laminariales with alternating gametophytic and sporophytic generations. On the coasts of Korea, sporophytes appear between October and November, and then grow rapidly from winter to spring when seawater temperatures are between 10 and 16°C (Lee and Sohn 1993; Oh and Koh 1996). Sporophytes of *U. pinnatifida* produce zoospores in spring from sporophylls positioned on the basal part of the stipe, and die off in summer with increasing temperature (Akiyama and Kurogi 1982; Lee and Sohn 1993; Oh and Koh 1996).

The growth of *U. pinnatifida* sporophytes occurs in the meristematic zone between stipe and blade, and its apical parts simultaneously degenerate (Castric-Fey et al. 1999b). In many kelps (e.g., *Laminaria hyperborea*, *L. setchelli*, *L. saccharina* and *Pterygophora californica*), the activity of the meristematic zone is regulated by photoperiod or endogenous clock (Lüning 1993). The reproduction of these kelps only takes place when the meristematic region is inactive for at least some time. Such a growth pattern makes it difficult to use plant length (or weight) as a growth character. Punched-hole and tagging methods have been applied to estimate the growth of *U. pinnatifida* sporophytes as has been done in kelp, but these methods are impractical because the frond is so thin and breakable in the field (Ishikawa 1993; Castric-Fey et al. 1999a,b). Thus, it is necessary to find a suitable morphological character to estimate the growth of *U. pinnatifida* sporophytes and to know whether the vegetative growth stops as reproduction occurs. These data are invaluable in commercial seaweeds for estimating total production and deciding the harvesting period of *Undaria* sporophytes.

Due to its ecological and commercial importance, numerous studies on the eco-physiology of *U. pinnatifida* have been carried out (Yamanaka and Akiyama 1993). Rope cultivation of *U. pinnatifida* was begun in 1964 in Korea. Thereafter, the annual production of *U. pinnatifida* was steadily increased up to 410,000 tons (in wet wt.) in 1994 (Shon 1998). However, there is little information on the growth and reproduction of *Undaria* sporophytes in a cultivation farm, even though these types of study are fundamental to an understanding of its population dynamics.

The aims of the present study were to examine the growth and reproduction cycles of *U. pinnatifida* in a cultivated population and to determine a reliable morphological character representing its growth and reproduction.

Materials and methods

Sporophytes of *U. pinnatifida* f. *distans* were collected biweekly or monthly in a cultivation farm located in Young-ho bay, Busan, Korea (35°07'N, 129°06'E) from December 1995 to March 1996. During the study period, salinity was between 31.5–32.2‰ and the average temperature of surface seawater was 12.15°C ± 2.17 (mean ± SD) and monthly average temperatures ranged between 10.15°C ± 0.56 in February 1996 and 15.56°C ± 1.15 in November 1995. Measurements of daily surface seawater temperatures and salinity over the experimental period were obtained from the Korea Institute of Ocean Science in Dongbaek Island, which is situated only 2.0 km from the sampling site.

The cultivation farm was established 50 m from the shore. General culture techniques of *U. pinnatifida* (e.g., spore seeding, culture of gametophytes, and transplantation) and a horizontal hanging single line method for field cultivation were used as described by Ohno and Matsuoka (1993). On 13 October 1995, the seedling lines with an approximate density of 3–5 plants cm⁻¹ rope and plants 1–2 cm long were transplanted onto the 20 horizontal ropes (3 cm in diameter), 100 m long. They were suspended at 1 m depth in the cultivation farm. All plants within 10 cm of cultivation ropes were collected using a knife on board a boat. At each collection, *U. pinnatifida* sporophytes were sampled from three different cultivation ropes in order to use replicates, and were transported to the laboratory.

Yield (g cm⁻¹ rope) and density (plants cm⁻¹ rope) for each replicate were determined. The growth of *U. pinnatifida* sporophytes was measured for 12 characters. Plants were weighed with a balance (CAS, Model ME-2100) after blotting with absorbent paper, and the thickness of the blades measured using a microscope after section. The number of pinnae were also counted, and the other 10 characters were as described in Figure 1. However, in the present study, plant lengths were not used for analysis because data

Figure 1 Morphometric measurements of *Undaria pinnatifida*; PL plant length; FL frond length; BW blade width; UBW undivided blade width; SW stipe width; SL stipe length; SPW sporophyll width; SPL sporophyll length; LSB length between sporophyll and blade; LSH length between sporophyll and holdfast.

were problematic due to erosion of apical blades. Growth was presented in absolute values over time and as relative growth rate (RGR), which was calculated for each mean of 11 characters using the equation:

$$\text{RGR} = (\ln P_2 - \ln P_1) / T_2 - T_1$$

in which P_1 and P_2 is the size of each morphological character at times T_1 and T_2 , respectively.

To determine a reliable morphological character representing the growth of *U. pinnatifida*, the relationships between weight and other growth characters were examined with all samples of 3 replicates ($n = 71$) collected on 13 December 1995. However, the relationship between fertility and blade width (or stipe width) of *Undaria* sporophytes was determined for 3 replicates at each collection. Fertility was the percentage of sporophyllous plants with undulations (or frills) for total plants. In general, sporophylls with undulations are fertile (Castric-Fey et al. 1993).

A one-way ANOVA was applied to test the effect of time on the growth of 11 characters in

Undaria sporophytes, and to discover whether there were differences in growth between fertile and non-fertile plants. Prior to analysis, homogeneity of variance was tested using Cochran's test and, where necessary, data were transformed. When significant differences between means were found, a Tukey's multiple comparison was applied (Sokal and Rohlf 1995). Statistical analyses were carried out using STATISTICA ver. 5.0.

Results

Yield and density

Mean yields of *Undaria pinnatifida* rapidly increased, whereas density decreased over the study period (Figure 2). The highest yield was 358 g cm^{-1} rope in March, whereas the least value with 93 g cm^{-1} rope was found on 13 December. Final yield was four times greater compared to initial collection (13 December) and a significant difference was found between monthly mean yields (ANOVA, $F_{1,5} = 25.43$, $p < 0.001$). Density decreased from 2.37 ± 0.28 (mean $\pm$ SE, $n = 3$ replicates) to 0.97 ± 0.03 plants cm^{-1} rope at the final collection and there was a significant difference between sampling dates (ANOVA, $F_{1,5} = 11.69$, $p < 0.001$).

Growth

Undaria sporophytes grew fast both in length and width resulting in an increase of mean plant weight during the survey period (Figure 3). Mean plant length was $79.06 \text{ cm} \pm 6.09$ (mean $\pm$ SE) in early December 1995 and reached $109.12 \text{ cm} \pm 2.79$ in March. On 13 December, the apical parts of blades were damaged by usual levels of wave action in the open sea. Thus, the plant length and frond length are the same in the present study. Mean plant weights ranged between $39.98 \text{ g} \pm 4.08$ and $371.82 \text{ g} \pm 35.32$ (mean $\pm$ SE) with minimal and maximal values in December and March, respectively. Also, blade width, number of pinnae and stipe width increased over time, and they were also greater at the final collection compared to the initial one. There were significant differences between collections for the weight, length, blade width, number of pinnae and stipe width of *Undaria* sporophytes (ANOVA, $p < 0.05$). However,

Figure 2 Variations in mean yield and mean density of *Undaria pinnatifida* during the study period. Bars show standard errors ($n=3$ replicate ropes).

stipe length and undivided blade width fluctuated and no significant differences were found between collections for both (ANOVA, $p > 0.05$).

Plant weight was positively correlated with 6 morphological characters: frond length, stipe length, stipe width, number of pinnae, blade width, and undivided blade width (Table 1). Plant weights were fitted well with stipe width ($R^2 = 0.89$, $n = 71$) and blade width ($R^2 = 0.90$, $n = 71$). Mean blade thickness of *U. pinnatifida* was $254.75 \mu\text{m} \pm 17.23$ (mean $\pm$ SE, $n=20$) in adult plants and breakable for wave action, but its stipe was strong and relatively thick ($8.70 \text{ mm} \pm 0.54$, $n = 20$).

Absolute growth values of all characters in *U. pinnatifida* sporophytes increased steadily over the survey period. However, the relative growth rates of *Undaria* for weight, length and blade width varied in the range of $-0.003 \sim 0.072 \text{ day}^{-1}$ (Table 2), while the growth patterns of each part were different. The RGRs of frond and sporophyll length (vertical growth parts) were greater than the RGRs of blade and sporophyll width (horizontal growth parts) from outplanting to 13 December 1995. After the middle of December, when 38% of sporophytes had matured, the growth almost stopped in a vertical direction, but horizontal growth (e.g., blade width, undivided blade width and stipe width) continued. In the middle of January, the growths in both vertical and horizontal directions were almost stopped with the maturation of *U. pinnatifida* sporophytes. At the end of February

1996, the weight of *U. pinnatifida* increased again by growth in width.

Sporophyll growth and fertility

Undaria sporophytes bearing sporophylls were found on 13 December 1995, 60 days after transplantation. At the initial collection, 27 sporophytes of 71 plants formed sporophylls without undulations. Over the monitoring period, the width and length of sporophylls increased from 2.55 cm to 7.07 cm and from 8.45 cm to 18.88 cm, respectively (Figure 3). Both were significantly greater in March compared to those of earlier collections (ANOVA, $p < 0.01$).

The relative growth of sporophylls declined rapidly after 23 December (Table 2). Higher growth rates of reproductive organs between 13 October and 13 December resulted from the initiation of sporophylls. Sporophylls grew both in length and width, even though vegetative growth rates were retarded. However, the length between sporophyll and blade (LSB) and between sporophyll and holdfast (LSH) showed negative growth.

Fertility of sporophytes was 38.02, 62.00, 72.46, 81.13, 95.35 and 96.55% at each collection time and gradually increased during the study period. Two plants in February and one plant in March 1996 remained immature. Fertile sporophytes were significantly greater in seven growth characters than non-fertile plants (Table 3).

Figure 3 Morphological variations for 11 characters of *Undaria pinnatifida* during the study period (arrow sporophyll formation, double arrow fertile sporophyll with undulations). Bars show standard errors ($n=3$ replicate ropes). Letters *a* to *d* indicate significant group of means found with the Tukey HSD tests.

Table 1 Relationship between plant weight and morphological character of *Undaria pinnatifida* ($n=71$)

Correlation	Equation
Weight vs frond length	$Y = 0.43x + 2.86, R^2 = 0.84$
Weight vs stipe length	$Y = 0.55x + 1.18, R^2 = 0.73$
Weight vs stipe width	$Y = 0.46x + 0.69, R^2 = 0.89$
Weight vs number of pinnae	$Y = 0.33x + 1.47, R^2 = 0.52$
Weight vs blade width	$Y = 0.54x + 1.67, R^2 = 0.90$
Weight vs undivided blade width	$Y = 0.28x + 0.90, R^2 = 0.47$

All data were Ln-transformed

Average stipe- and blade- widths were positively correlated with fertility of *U. pinnatifida* sporophytes (Figure 4). Fertility fitted better with stipe width ($R^2 = 0.97$) than blade width ($R^2 = 0.81$). Also, plants with stipe widths were <5 mm did not produce sporophylls, but all plants with stipe widths >21 mm were fertile.

Discussion

Plant weight of *Undaria pinnatifida* sporophytes increased steadily over the experimental period in four different ways. It increased by both vertical and horizontal growth (October–early December), and by horizontal growth with the thickening of blade and stipe when sporophytes began to be fertile (December–January). In the middle of January, vertical and horizontal growth had almost stopped with maturation of *U. pinnatifida* sporophytes. Finally, the weight of

U. pinnatifida increased again by horizontal growth at the end of February. Present results indicate that the plant weight of *U. pinnatifida* increases by vertical and horizontal growth at different times, and reproduction occurs when vegetative growth has stopped. Thus, the harvesting period of *U. pinnatifida* sporophytes should be in January during the luxuriant growth period before all plants are fertile.

The growth and reproduction of the kelp *U. pinnatifida* depend on the activity of the meristematic zone, located between stipe and blade. When the meristematic zone is very active, the vertical elongation and horizontal expansion of *Undaria* sporophytes occur and plant weight increases rapidly. However, if sporophytes start to form sporophylls, the meristematic region becomes inactive; the vegetative growth was retarded or stopped in the present study. In kelps, the activity of the meristematic zone is usually regulated by photoperiod or endogenous clocks (Lüning 1993). The sporophytes of *Pterygophora californica* have a circannual growth rhythm related to an annual cycle of daylength, with other kelps and sorus production of kelps also controlled by photoperiod in *Laminaria saccharina* and *L. setchelli* (Lüning 1988, 1993). Sporophyll formation of *U. pinnatifida* sporophytes was more enhanced under a long-day than under a short-day regime (Pang and Lüning 2004). Thus, the *U. pinnatifida* sporophyte is a facultative long-day plant in regard to sporophyll formation, but this is different from *U. pinnatifida* gametophytes which are almost obligate short-day plants in egg production and fertilization (Choi et al. 2005).

Table 2 Variations in relative growth rates for 11 morphological characters of *Undaria pinnatifida* over the study period

Characters	13 Dec ($n=71$)	23 Dec ($n=50$)	16 Jan ($n=69$)	26 Jan ($n=53$)	23 Feb ($n=43$)	11 Mar ($n=29$)
Weight	0.061	0.041	0.011	0.031	0.019	0.045
Frond length	0.072	0.004	0.006	0.002	−0.003	0.012
Blade width	0.060	0.020	0.004	0.018	0.011	0.026
Undivided blade width	0.032	0.008	0.009	0.026	−0.005	0.006
Pinnate number	0.044	0.014	0.003	0.000	0.002	0.001
Stipe length	0.053	0.007	0.006	−0.001	−0.002	0.004
Stipe width	0.039	0.015	0.008	0.011	0.006	0.002
Sporophyll length	0.074	0.006	0.011	0.026	0.006	0.004
Sporophyll width	0.054	0.023	0.003	0.019	0.010	0.017
LSB	0.165	−0.010	0.001	−0.037	−0.020	0.015
LSH	0.130	0.020	0.014	−0.009	−0.011	−0.009

LSB Length between sporophyll and blade; LSH length between sporophyll and holdfast

Table 3 Results of one-way ANOVA test for fertile and nonfertile plants on seven characters in *Undaria pinnatifida*

Factors	Fertile (<i>n</i> = 220)	Non-fertile (<i>n</i> = 95)	<i>p</i>	Transformation
Wet weight (g)	150.3 ± 9.90	22.4 ± 1.67	<0.001	Ln +1
Length (cm)	102.2 ± 1.67	62.5 ± 2.57	<0.001	None
Number of pinnae	19.3 ± 0.38	12.0 ± 0.38	<0.001	None
Stipe length (cm)	30.9 ± 0.74	18.51 ± 0.96	<0.001	None
Stipe width (mm)	17.0 ± 0.31	7.9 ± 0.30	<0.001	None
Blade width (cm)	70.1 ± 2.31	25.1 ± 1.26	<0.001	Ln
Undivided blade width (cm)	10.7 ± 0.32	5.3 ± 0.23	<0.001	Ln

Data were analyzed for all plants collected during the study period

Although plant weight is a representative growth character, the growth estimation of *Undaria* sporophytes is not easy because of the growth pattern (Castric-Fey et al. 1999b). Thus, the length (or weight) of *Undaria* sporophytes cannot be used as a suitable growth character. The punched-hole method for adults, 75 cm long (Ishikawa 1993; Yoshikawa

et al. 2001), and tagging method for juveniles (Castric-Fey et al. 1999b), have been used for growth measurement in *U. pinnatifida*. Both methods are certainly useful for measuring the elongation of individual fronds in *U. pinnatifida*, and in particular the erosion rate of blades can be estimated by the punched-hole method (Ishikawa 1993; Castric-Fey

Figure 4 Relationship between fertility and (a) stipe width or (b) blade width of *Undaria pinnatifida* during the study period. Bars show standard errors (*n* = 3 replicate ropes).

et al. 1999b). However, the punched-hole method cannot be applied to smaller plants and requires laborious work (measuring the distance between punched holes and punching a new hole weekly or biweekly), which is difficult in the sublittoral zone in which *U. pinnatifida* grows. Furthermore, the blades of *U. pinnatifida* are so thin (150–400 µm) and breakable compared to *Laminaria* that growth cannot be measured by this method (Castric-Fey 1999a). The tagging method is also problematic because of the loss of tagged labels, damage to juvenile plants by tagging and the requirement to use the punched-hole method when it starts to degenerate. Also, both methods only estimate vertical growth but not horizontal (or marginal) growth. Castric-Fey et al. (1999b) reported that midrib width is measurable for both juvenile and adult plants and that it is a persistent character representing the growth of *U. pinnatifida* even during the disintegration of blades. However, juvenile sporophytes (1–10 cm in blade length) of *U. pinnatifida* have no midrib (Castric-Fey et al. 1999b). Thus, we suggest that stipe width is the most feasible morphological character with which to estimate the growth and reproduction of *U. pinnatifida*, because stipe width was positively correlated and fitted well with plant weight and fertility of *Undaria* sporophytes.

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