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Research paper

Assessing establishment success of *Zostera marina* transplants through measurements of shoot morphology and growth

Wen-Tao Li [a,b], Jong-Hyeob Kim [a], Jung-Im Park [a], Kun-Seop Lee [a,*]

[a] *Department of Biological Sciences, Pusan National University, 609-735, Busan, Republic of Korea*
[b] *Department of Marine Fisheries, Ocean University of China, Qingdao 266003, PR China*

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A B S T R A C T

Since significant seagrass declines have been reported worldwide, numerous seagrass restoration projects through transplantation have been attempted in recent decades. In this study, *Zostera marina* shoots were transplanted into Jindong Bay on the southern coast of Korea in November 2006 to assess establishment success of the transplants to a new transplant environment. Shoot density, individual shoot weight, productivity, and morphological characteristics of transplants and reference plants in the vicinity of the planting site were monitored monthly for 13 months. Although shoot size of transplants was smaller than that of reference plants at the start of transplantation, individual shoot weight, leaf width, shoot height, and rhizome diameter of transplants increased rapidly, reaching even higher values than those of reference plants 5 months after transplantation. These results suggest that eelgrass transplants established morphologically 5 months after transplantation. Shoot productivity of transplants was lower than that of reference population during the first 5–6 months following transplantation, but became higher than that of reference population 6 months after transplantation. The higher transplant productivity was likely due to the lower shoot density at the transplant site than that at the reference population. Rapid changes in shoot morphology and growth of transplants indicated that eelgrass transplants had great morphological plasticity and established successfully in the new environment within 5–6 months. In addition to survival rates of transplants, monitoring of shoot morphology and growth appeared to be an effective approach for accurate assessment of the establishment success of eelgrass transplant.

1. Introduction

Although seagrass is an important component in coastal and estuarine ecosystems, seagrass meadows have suffered extensive losses for decades due to natural and anthropogenic causes in the global context (Short et al., 1991; Boström et al., 2002; Hauxwell et al., 2003; Burkholder et al., 2007; Montefalcone et al., 2010). Transplantation of seagrasses has been considered to be an effective method to mitigate and control seagrass degradation (Davis and Short, 1997; Orth et al., 1999; Fishman et al., 2004; Paling et al., 2007; Park and Lee, 2007; Bastyan and Cambridge, 2008). Several transplanting methods have been developed, and numerous attempts at transplantation have been conducted in the last decades (Davis and Short, 1997; Orth et al., 1999; Park and Lee, 2007). A few seagrass transplantation trials have also been conducted in Korea in recent years, especially for the eelgrass *Zostera marina*, the most widely distributed species on the coasts of Korea (Park and Lee, 2007; Lee and Park, 2008).

Seagrass transplants usually show high mortality during the initial period following transplantation due to initial short-term stress resulting from injuries, desiccation, and impaired function during the planting process, after which the survived transplants establish at the new site (Zimmerman et al., 1995; van Tussenbroek, 1996; Worm and Reusch, 2000; Paling et al., 2001a; Meehan and West, 2002; Park and Lee, 2007; Lee and Park, 2008; Milbrandt et al., 2008). Because transplants are adversely affected by transplantation in terms of their morphology and physiology during the initial period after transplantation, the initial stress can be evaluated by measuring either physiological or morphological changes (Zimmerman et al., 1995; Moore et al., 1996; van Tussenbroek, 1996; Horn et al., 2009). Although numerous studies on seagrass transplantation have been conducted around the world, most have mainly focused on the transplantation methods, planting seasons, and survival rates of transplants under different environmental conditions (Zimmerman et al., 1995; Moore et al., 1996; Orth et al., 1999; Paling et al., 2001b; Bastyan and Cambridge, 2008). Several

* Corresponding author.
E-mail address: klee@pusan.ac.kr (K.-S. Lee).

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studies also measured phenological characteristics, morphology, and growth of seagrass transplants after transplantation (Zimmerman et al., 1995; Fonseca et al., 1996; Paling et al., 2007; Cambridge and Kendrick, 2009). However, few studies have dealt with the establishment processes of seagrass transplants using continuous measurements of these parameters. Additionally, monitoring of seagrass transplants was usually conducted without comparison with reference plants. The lack of comparative data has inhibited the development of criteria for evaluating transplant success and understanding the establishment processes of such transplants (Fonseca et al., 1996).

In this study, we measured shoot morphology and growth at both the transplant site and the reference population, which was a natural eelgrass population in the vicinity of the transplant site, for over a year. The physiological status of the transplants can be accurately assessed through comparing shoot morphology and growth of transplants and reference population in similar environmental conditions. The objective of this study was to investigate the establishment success of seagrass transplant to the new transplant environment. We hypothesized that the shoot morphology and growth of *Z. marina* transplants would be reduced initially due to the initial transplanting stress and the shoots would then establish in the new area with shoot morphology and growth similar to reference population after a certain period of time.

2. Materials and methods

2.1. Study sites

The transplant site was located in Jindong Bay (35°05.6′N, 128°33.6′E) on the southern coast of Korea (Fig. 1). *Zostera marina* was once widespread in this site, but most eelgrass shoots disappeared from the site due to the seashore road construction. Some eelgrass patches were present in the vicinity of the planting site. Thus, environmental conditions at this site are probably being restored to the original conditions. The eelgrass shoots in the vicinity of the transplant site was used as reference plants (Fig. 1). Donor shoots used for transplantation were collected from Koje Bay (34°48.0′N, 128°35.0′E) about 33 km south of the transplant site (Fig. 1). The sediment of the donor bed was characterized by a high sand content, whereas the transplant site had loam sediment. The donor bed was located in an intertidal area, whereas the transplant site was located in a subtidal area with water depth of about 1.0 m below MLLW.

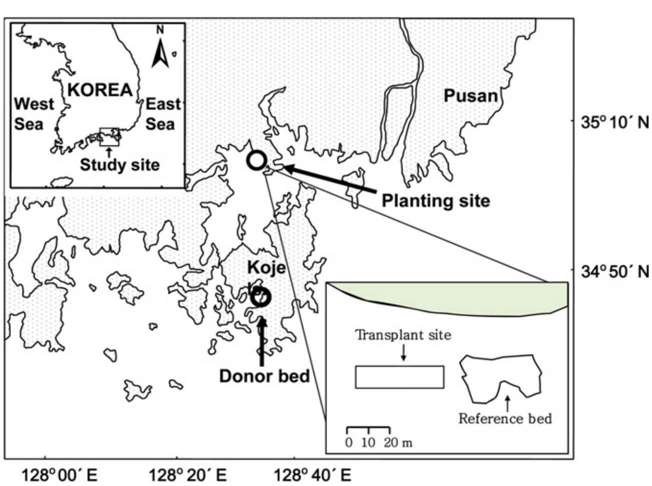

Fig. 1. Transplant site and donor bed on the southern coast of Korea.

2.2. Seagrass transplantation

Eelgrass transplantation was conducted in November 2006. Vegetative shoots used for transplantation were collected individually by hand to minimize damage to the donor bed. Special care was taken to avoid damage or loss of below-ground tissues. Intact eelgrass shoots were selected, immersed in seawater to avoid desiccation, and transplanted within 24 h. Eelgrass plants were hand-planted by Scuba divers using the staple method, in which two eelgrass shoots were attached to one V-shaped metal staple and anchored to the sediment (Davis and Short, 1997; Fonseca et al., 1998; Park and Lee, 2007). Sixteen planting units (i.e., 32 shoots) were planted in each 1×1 m plot, and 70 plots were planted in the transplant site. After transplantation, monitoring of transplants and reference plants was conducted at both the transplant site and the nearby reference bed for 13 months, from November 2006 to December 2007.

2.3. Physiochemical parameters

Underwater photosynthetic photon flux density (PPFD) and water temperature at the eelgrass canopy level were monitored continuously every 15 min throughout the study period using HOBO data loggers (Onset Computer Corp.) encased in a waterproof underwater housing. Measured water temperature was averaged daily. Light intensity (lumens ft^{-2}) measured using the HOBO data logger was converted to PPFD (μmol photons m^{-2} s^{-1}) by concurrent quantum measurements using an LI-1400 data logger and an LI-193SA spherical quantum sensor (Li-Cor, Inc.). A regression analysis was performed to convert the light intensity to PPFD ($y = 9.395\,x + 4.564$, $r^2 = 0.84$). Daily PPFD (mol photons m^{-2} d^{-1}) was calculated as the sum of the quantum flux over each 24-h period.

To determine water column inorganic nutrient (NH_4^+, $NO_3^- + NO_2^-$, and PO_4^{3-}) concentrations, four replicate surface water samples were collected monthly using 150-ml bottles. Sediment pore water nutrients were measured monthly from four replicate sediment samples, which were collected haphazardly to a sediment depth of about 13 cm using a sediment corer. Water and sediment samples were stored on ice for transport to the laboratory. Sediment pore water was extracted by centrifugation (5000*g* for 15 min) and then diluted with low-nutrient seawater (<0.1 μM) for determination of pore water nutrient concentrations. Water samples of both water column and sediment were analyzed using standard colorimetric techniques following the methods of Parsons et al. (1984).

2.4. Biological measurements

Shoot density, morphology, and individual shoot weight of transplants and reference plants in the vicinity of the transplant site were monitored monthly throughout the experimental period. Shoot density was estimated by counting the number of shoots inside a haphazardly placed quadrat (0.5×0.5 m; $n = 4$), and the measurements were converted to shoots per unit area values (shoots m^{-2}).

Shoot morphology was measured using terminal shoots of both transplants and reference population. For morphological measurements, five transplants and 12 reference plants were haphazardly collected, washed with tap water in the laboratory, and thoroughly cleaned of epiphytes and sediment. The number of leaves was counted from the upper end of the sheath, and shoot height was measured from the meristem to the longest leaf tip. Leaf width at the middle of the longest leaf was measured to the nearest 0.1 mm. Rhizome diameter of each internode (from the first to the sixth internode, counted from the meristem) was also measured to nearest 0.1 mm. The average diameter along the six internodes was used to represent rhizome diameter. After morphological measurements, all shoot samples were used to estimate the individual shoot weight. All tissues of plant samples were dried at 60 °C for 48 h to obtain above- and below-ground tissue weight.

2.5. Above- and below-ground productivities

Above- and below-ground productivities were estimated using the plastochrone method (Jacobs, 1979; Short and Duarte, 2001; Gaeckle and Short, 2002). Ten to 12 haphazardly chosen shoots were marked through the sheath bundle about 3 cm above the meristem using a hypodermic needle. After 4–5 weeks, the marked shoots were retrieved for productivity assessment. Plastochrone intervals were calculated by dividing the marking period (in days) by the number of new leaves produced after marking. The dry weight of the youngest mature leaf, which was usually the third leaf, and the mature rhizome/root segments, which had the largest diameter in the first to sixth youngest internodes, were measured every sampling time. The above- and below-ground productivities of each shoot were calculated using the following equations:

$$\text{Above}-\text{ground productivity}\left(\text{mg DW shoot}^{-1}\ \text{day}^{-1}\right) = \frac{\text{dry weight of a mature leaf}\left(\text{mg DW shoot}^{-1}\right)}{\text{plastochrone interval (day)}}$$

$$\text{Below}-\text{ground productivity}\left(\text{mg DW shoot}^{-1}\ \text{day}^{-1}\right) = \frac{\text{dry weight of a mature rhizome/root segment}\left(\text{mg DW shoot}^{-1}\right)}{\text{plastochrone interval (day)}}$$

2.6. Statistical analyses

All values are reported as mean ± standard error. Statistical analyses were performed using SPSS 16.0 (SPSS Inc., Chicago, Illinois, USA). Data were tested for normality and homogeneity of variance to meet the assumptions of parametric statistics. If these assumptions were not satisfied, data were log transformed. Significant differences in underwater irradiance, water temperature, and water column and sediment nutrient concentrations among sampling months were tested using a one-way analysis of variance (ANOVA). Differences in shoot density, morphology, individual shoot weight, and productivity among sampling months and between transplants and reference population were tested for significance using a two-way ANOVA and a post-hoc analysis.

3. Results

3.1. Environmental parameters

The underwater irradiance exhibited a clear temporal variation (Fig. 2A). PPFD was highest during winter and early spring and lowest during fall, although exceptionally high irradiance values were also observed in June 2007. PPFD was less than 1.0 mol photons m^{-2} d^{-1} for approximately 10 consecutive days during late October and early November 2007. Water temperature also showed an obvious temporal variation pattern (Fig. 2B), with the lowest value of 6.4 °C in January 2007, and the highest value of 29.9 °C in August 2007. Monthly average of the underwater irradiance and water temperature changed significantly ($p < 0.001$) with sampling months.

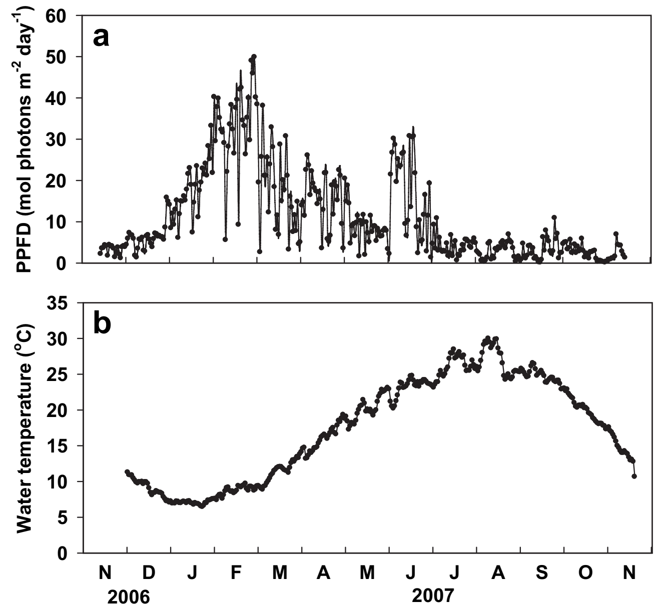

Fig. 2. Temporal changes in (a) underwater photosynthetic photon flux density (PPFD) and (b) temperature during the experimental period from November 2006 to December 2007.

Water column inorganic nutrients, NH_4^+, $NO_3^- + NO_2^-$, and PO_4^{3-} concentrations, were of the same order of magnitude, and showed significant ($p < 0.001$) temporal variations. Water column NH_4^+ concentration was highest (4.9 μM) in June 2007 and lowest (0.6 μM) in December 2006 (Fig. 3A). $NO_3^- + NO_2^-$ concentration was highest (7.3 μM) in December 2006, and lowest (0.6 μM) in June 2007 (Fig. 3B), showing a variation pattern nearly opposite that for NH_4^+ concentration. Water column PO_4^{3-} concentration changes significantly ($p < 0.001$) with sampling months, but did not show clear seasonal variation pattern, with highest (1.6 μM) in November 2006 and lowest (0.5 μM) in December 2007 (Fig. 3C).

The sediment pore water nutrient concentrations also changed significantly ($p < 0.001$) with sampling months, but did not show clear seasonal variation patterns (Figs. 3D, 3E, 3F). Concentrations of sediment pore water NH_4^+ and $NO_3^- + NO_2^-$ were one or two orders of magnitude higher than those in the water column, whereas PO_4^{3-} concentration was the same order of magnitude as the water column. NH_4^+ concentration was highest (248.3 μM) in January and lowest (66.2 μM) in August 2007. For $NO_3^- + NO_2^-$ concentration, the highest value (77.1 μM) occurred in January, and the lowest (14.2 μM) occurred in May 2007. The sediment pore water PO_4^{3-} concentration was about four times higher than in the water column, and it was lowest (3.2 μM) in July 2007 and highest (6.3 μM) in December 2007.

3.2. Shoot density and individual shoot weight

The shoot densities of reference population and transplants changed significantly ($p < 0.001$) with sampling months. The shoot

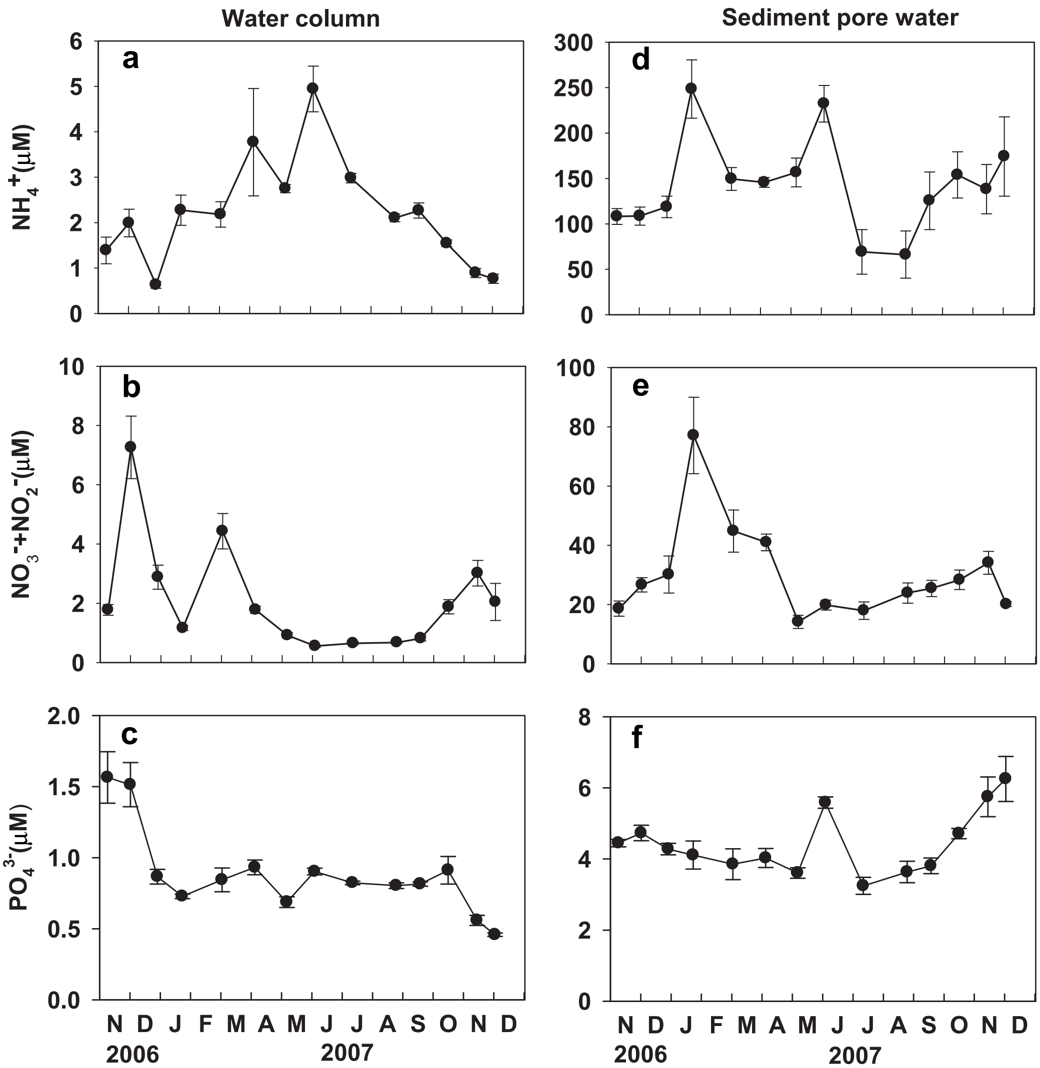

Fig. 3. Temporal changes in (a) water column NH4$^+$, (b) NO3$^-$ + NO2$^-$, (c) and PO4^{3-} concentrations, and sediment pore water (d) NH4$^+$, (e) NO3$^-$ + NO2$^-$, (f) and PO4^{3-} concentrations from November 2006 to December 2007. Vertical error bars show the standard error of measured values.

density of reference population was highest (202 shoots m^{-2}) in May and lowest (82 shoots m^{-2}) in November 2007, whereas that of transplants was highest (88 shoots m^{-2}) in August 2007 and lowest (46 shoots m^{-2}) in December 2006. Transplant shoot density increased gradually without an initial decline until August 2007, and then slightly decreased during fall 2007. Although eelgrass shoots were transplanted in the transplant site at much lower shoot density than that of the reference population, shoot density in the transplant site approached that of the reference population by August 2007.

The above-ground tissue weight of individual transplants was significantly lower ($p < 0.05$) than that of reference plants for the first 5 months post-transplanting (Fig. 4B). After that period, the weight of transplants was usually significantly higher ($p < 0.05$) than that of reference plants. The highest above-ground tissue weight of both transplants and reference population occurred in June 2007, whereas the lowest values of transplants and reference population occurred in November 2006 and May 2007, respectively. The below-ground tissue weight exhibited a similar variation pattern to that of above-ground tissues, but the weight was far less than that of above-ground tissues. The below-ground tissue weight of transplants was significantly lower ($p < 0.05$) than that of reference plants for the first 2–3 months after transplanting and then increased rapidly, becoming higher significantly ($p < 0.01$) than that of reference population by May 2007 (Fig. 4C). The total individual shoot weight showed a similar trend to the above- and below-ground tissue weights for both transplants and reference population (Fig. 4D). Thus, the shoot weight of transplants became comparable to, or even higher than, that of reference plants about 4–6 months after transplantation.

3.3. Shoot morphology

The number of leaves in transplants was significantly ($p < 0.05$) less than that of reference plants during the first 5 months after transplantation, except in December 2006, but was significantly ($p < 0.01$) more than that of reference plants from May 2007. The number of leaves in reference population showed a clear temporal variation, with lowest values (3.4 leaves shoot^{-1}) in August 2007 and highest values (8.0 leaves shoot^{-1}) in March 2007 (Fig. 5A). Leaf width of transplants was significantly ($p < 0.01$) less than that of reference plants for the first 3 months after transplantation, but increased rapidly and became even wider ($p < 0.01$) than that of reference population from May 2007 (Fig. 5B). The shoot height of

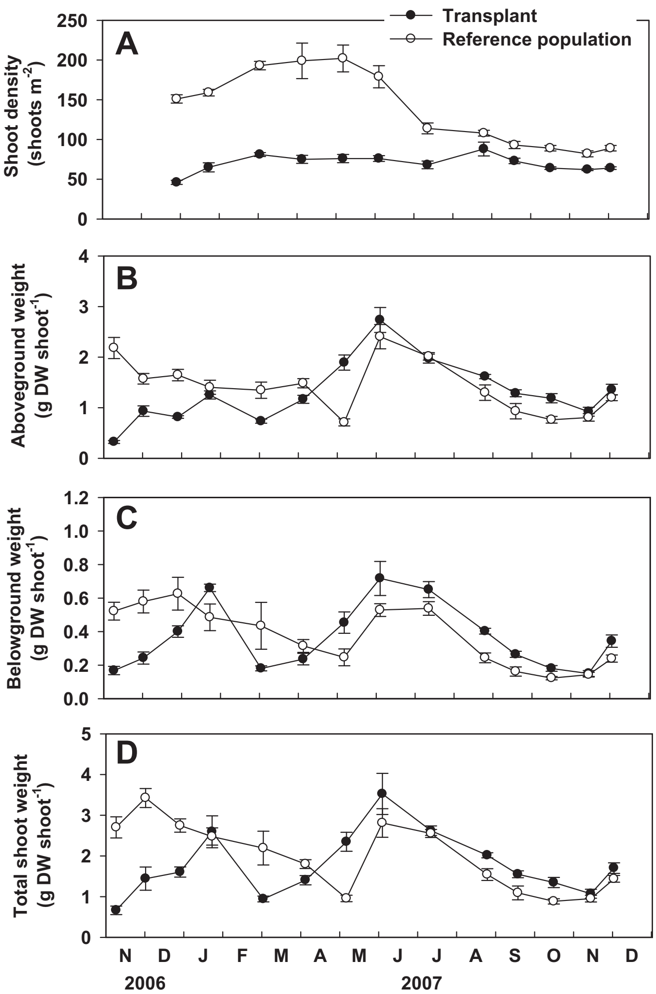

Fig. 4. Shoot density (A), above-ground weight (B), below-ground weight (C), and total weight (D) of individual transplants and reference plants after transplantation from November 2006 to December 2007. Vertical error bars show the standard error of measured values.

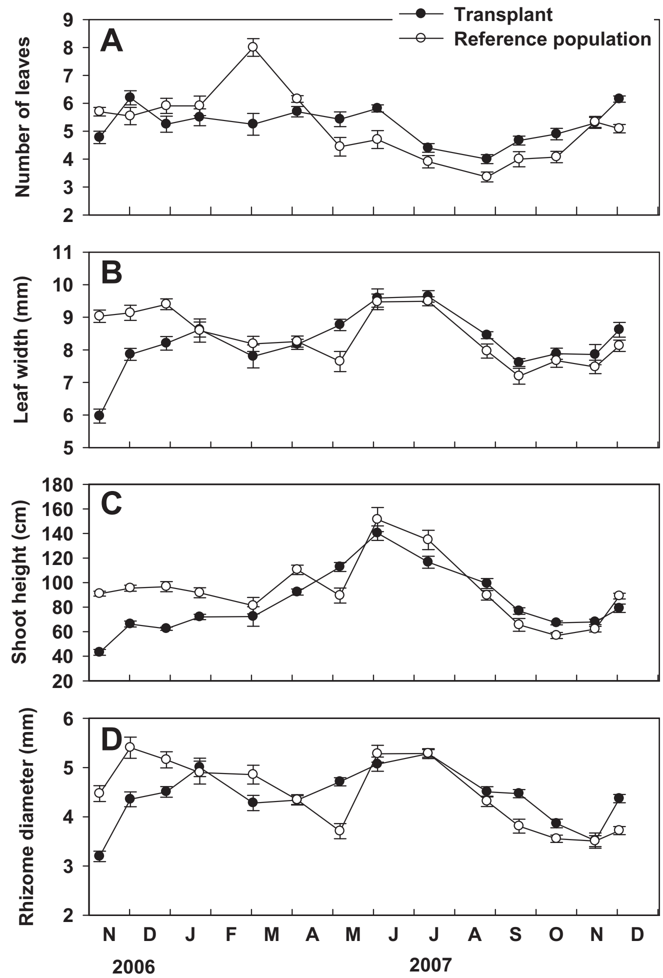

Fig. 5. Morphological characteristics of transplants and reference plants during the experimental period from November 2006 to December 2007. Numbers of leaves (A), leaf width (B), shoot height (C), and rhizome diameter (D). Vertical error bars show the standard error of measured values.

transplants was significantly ($p < 0.01$) less than that of reference plants for the first 4 months ($p < 0.01$), and then became similar ($p = 0.602$) to that of reference population, showing similar temporal variation patterns (Fig. 5C). The rhizome diameter of transplants was significantly ($p < 0.05$) narrower than that of reference plants for the first 3 months after transplantation, and then became similar ($p = 0.148$) to that in reference population (Fig. 5D).

3.4. Above- and below-ground productivity

The above-ground productivity of transplants was slightly lower than that of reference plants for the first 3 months after transplantation (Fig. 6A). However, the above-ground productivity of transplants has become significantly higher ($p < 0.05$) than that of reference plants by March 2007 (i.e., ca. 4 months after transplantation). The above-ground productivity showed significant ($p < 0.001$) temporal variations in both transplants and reference population (Fig. 6A). The highest above-ground productivity occurred in summer (60.9 mg DW shoot^{-1} d^{-1} in June for transplants and 36.2 mg DW shoot^{-1} d^{-1} in July for reference population) and the lowest values occurred in winter (12.6 mg DW shoot^{-1} d^{-1} in January for transplants and 9.1 mg DW shoot^{-1} d^{-1} in February for reference population; Fig. 6A).

The below-ground productivity showed a similar variation to above-ground productivity, but took about 2 months longer than the above-ground productivity to reach similar or higher productivities to reference population (Fig. 6B). The below-ground productivity of transplants was significantly ($p < 0.01$) lower than that of reference plants for the first 5 months after transplantation, but was also significantly higher ($p < 0.01$) than reference plants from May 2007 (Fig. 6B). Because the above-ground productivity accounted for approximately 79% and 75% of total productivity of transplants and reference population, respectively, variation in total productivity was closely correlated with variation in above-ground productivity (Fig. 6C). The total productivity of transplants was slightly lower than that of reference plants for the first 3 months after transplantation, but became significantly higher ($p < 0.05$) than that of reference plants after May 2007.

4. Discussion

4.1. Adaptation of transplants to the new environment

When transplanting seagrasses, the root and rhizome tissues are inevitably severed; thus, the survival and establishment of transplants can be reduced by damage to below-ground tissues (Fonseca

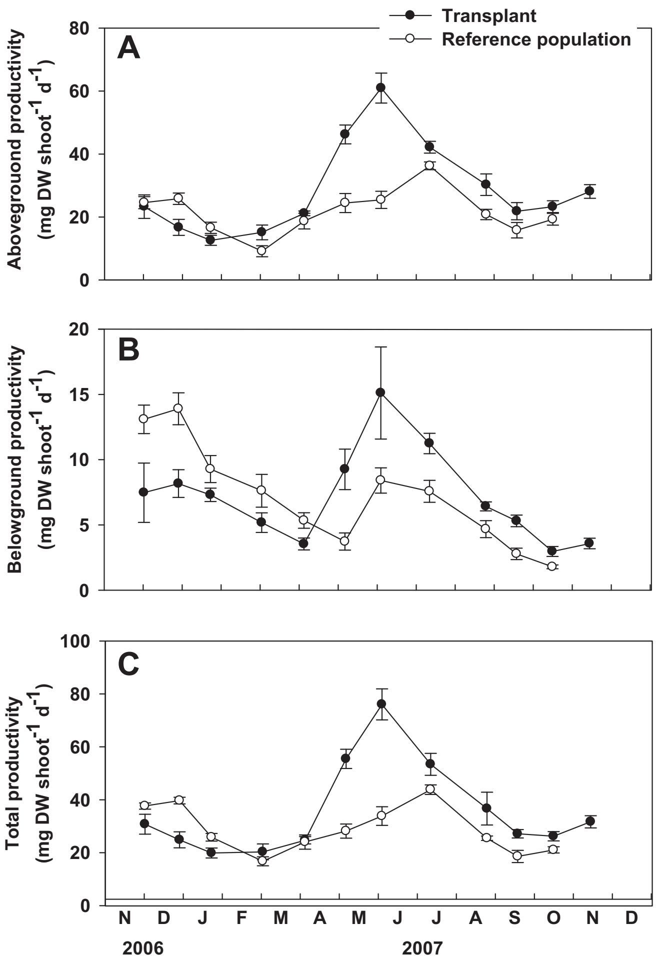

Fig. 6. Productivity of transplants and reference plants during the experimental period from November 2006 to December 2007. Above-ground productivity (A), below-ground productivity (B), and total productivity (C). Vertical error bars show the standard error of measured values.

et al., 1998). Sediment-associated seagrass transplanting methods (i.e., plug, sod, or core methods) are presumably less affected by the initial transplanting stress because of minimal disturbance to the rhizosphere (Fonseca et al., 1998). However, bare-root methods such as the staple method, TERFS, or shell method are the most used due to convenience in practice (Fonseca et al., 1996; Calumpong and Fonseca, 2001; Short et al., 2002; Lee and Park, 2008; Cambridge and Kendrick, 2009). The initial planting stress resulting from injuries or desiccation during the planting process usually occurs in the early period after transplantation, causing physiological and morphological changes in transplants (South and Zwolinski, 1997; Struve et al., 2000). The photosynthetic efficiency of *Posidonia sinuosa* transplants decreased during the first 1–2 months due to the initial transplanting stress (Horn et al., 2009). Leaf length and width also suddenly decreased after transplantation due to the initial planting stress in *Thalassia testudinum* (van Tussenbroek, 1996). However, in the present study, we did not observe declines in growth and morphology of transplants caused by the initial transplanting stress during the early months after transplantation. The lack of transplant responses to the initial planting stress might be due to phenotypic plasticity of the transplants. Plants possess a remarkable capacity to alter their phenotype in response to heterogeneous environmental conditions commonly encountered and display plastic responses to a wide variety of environmental conditions, such as light, temperature, nutrients, etc. (Sultan, 2000; Valladares et al., 2002; Callaway et al., 2003). Physiological and morphological variations are believed to be essential for the survival of plants in heterogeneous environments (Valladares et al., 2002). In this study, the donor bed was characterized by a high sand content in the sediment, with low pore water nutrient content, whereas the transplant site had loamy sediments with a high nutrient content (Park and Lee, 2007). Thus, reference plants in the vicinity of the transplant site were much bigger than the initial transplants from the donor bed. Because the small donor shoots, which grew at a site with low-nutrient availability, were transplanted to a high nutrient site, growth and shoot size of the transplants increased without initial declines during the early period of transplantation.

Light might also have played a role in the morphological changes of transplants in this study. Some researchers have found that plant leaves tended to be smaller under high light conditions (Witkowski and Lamont, 1991; Bintz and Nixon, 2001). The donor plants for transplantation were collected from an intertidal area, where the shoots were occasionally exposed to air; hence, the incident irradiance at canopy level was high, and thus the initial shoot size of transplants was smaller than that of reference plants, which were located in a subtidal area. After the transplants established in the new environment, the shoot size of transplants became similar to that of reference population.

Leaf width is considered to be an indicator of environmental stress (Phillips and Lewis, 1983), although it was shown to be less plastic than leaf length (van Tussenbroek, 1996). A reduction in leaf width with increasing duration of air exposure at low tide was recorded in *Halodule wrightii* (McMillan and Phillips, 1979). In this study, along with other morphological characteristics, leaf width increased greatly during the initial period after transplantation and became comparable to, or even greater than, that of reference population. The increase in leaf width of transplants probably resulted from release from the stress of air exposure in the donor beds and the exposure to high nutrient conditions in the planting site.

Seagrass biomass and productivity are generally affected by light conditions (Duarte, 1991; Peralta et al., 2002; Lee et al., 2007). In the present study, light was significantly reduced in May and July–November 2007 (Fig. 2A). The reduced light conditions might be an important cause of a reduction in biomass and productivity of both transplants and reference population during that period. Plant productivity is usually positively correlated with the amount of photosynthetic tissues (Duarte, 1989; Niklas and Enquist, 2001). In this study, productivities of transplants were significantly lower than those of reference population during first 5–6 months following transplantation. The lower transplant productivities were probably caused by the smaller transplant shoot size during the early period of transplantation. After transplants established at the planting site, their shoot weight and productivity became comparable to, or even higher than, those of reference population.

Plant density has been recognized as a major factor determining the degree of resource competition between plants (Gopal and Goel, 1993; Hashemi et al., 2005). In *Z. marina*, shoot weight and productivity per shoot were negatively correlated with shoot density (Duarte, 1989; Olesen and Sand-Jensen, 1994). Decreased productivity per shoot with increased plant density has also been found in many other plants (Retuerto et al., 1996; Hashemi et al., 2005). In this study, the shoot density in the transplant site was lower than that in the reference site for the first 10 months after transplantation. Thus, the transplants should have been less affected by the self-shading and competition for nutrient resources during this period. The reference site may have had intensified mutual shading and nutrient competition due to the higher shoot

Table 1
Summary of recent *Zostera marina* transplantation attempts conducted around the world.

Location	Monitoring parameters	Time for establishment (months after transplantation)	Planting method	References
San Francisco Bay, USA	Survival rate, growth, photosynthesis	12.0	Core	Zimmerman et al., 1995
Dutch Wadden Sea	Survival rate and development	ND	NM	van Tussenbroek, 1996
York River estuary, USA	Survival rate	ND	NM	Moore et al., 1996
York River estuary, USA	Survival rate	ND	NM	Moore et al., 1997
Dutch Wadden Sea	Shoot and biomass development; reproduction	ND	NM	van Katwijk et al., 1998
Chesapeake Bay, USA	Survival rate	8.0	DI	Orth et al., 1999
Netherlands	Growth and photosynthesis	ND	Pot	Peralta et al., 2003
Chesapeake Bay, USA	Success of planting and survival rate	6.0	DI, manually or mechanically	Fishman et al., 2004
Korea	Survival rate and establishment	1.0–2.3	Staple	Park and Lee, 2007
Korea	Survival rate and establishment	2.2–2.6	TERFS	Park and Lee, 2007
Korea	Survival rate and establishment	3.2–3.7	Shell	Park and Lee, 2007
Chesapeake Bay, USA	Seedling establishment	ND	Seed	Orth et al., 2009
Korea	Productivity and morphology	ND	Staple	Park et al., 2009

NM = not mentioned; DI = direct insertion method; ND = no data.

density. Thus, higher productivity in transplants than in reference population after 5–6 months following transplantation might be a result of less competition for light and nutrients in the transplant site.

4.2. Time for transplant establishment

The most widely used parameter in assessing establishment success of seagrass transplant is the survival rate of transplants (Table 1). During the early period after transplantation, transplant shoot density usually declines due to the initial transplanting stress and then increases through development of lateral shoots after establishment (Orth et al., 1999; Park and Lee, 2007; Bastyan and Cambridge, 2008; Lee and Park, 2008). Thus, an increase in transplant density has been used as an indicator of transplant establishment success in a transplant site. In the present study, however, the shoot density of the transplants did not decline during the early period of transplantation, and thus transplant establishment success could not be assessed by transplant survival rate or shoot density. We also monitored shoot morphology and growth to examine establishment success of seagrass transplants in this study. Additional monitoring of shoot morphology and growth as well as shoot density and survival rate provided valuable information for assessment of seagrass transplant establishment.

Comparing morphological and physiological characteristics of transplants and reference plants can lead to the development of criteria for assessing transplant establishment success (Balestri et al., 1998; Lee and Park, 2008). Seagrass shoot morphology and growth exhibit seasonal and spatial variations, and thus changes in transplants should be compared with those of reference population. In this study, we examined the establishment processes of transplants through measurements of shoot morphology and growth of both transplants and reference population. Based on these measurements, we concluded that transplants established successfully in the transplant site 5–6 months after transplantation. This establishment time was slightly longer than that obtained by Park and Lee (2007), who reported that eelgrass transplants planted using three planting methods required 1–4 months for establishment. The differences in establishment time were probably caused by use of the different criteria for assessing transplant establishment. In Park and Lee (2007), the establishment time was derived from shoot density and survival rate, whereas in this study, it was estimated based on transplant morphology and growth.

Below-ground tissues are important carbohydrate storage organs that enable perennial plants to support a rapid spring flush of leaf growth as well as leaf growth during adverse conditions (Zimmerman and Alberte, 1996; Burke et al., 1996). The increase in below-ground biomass of transplants probably resulted from accumulation of photosynthate in rhizomes. In this study, below-ground tissues of transplants required 2 months more than above-ground tissues (i.e., 6 months) to increase productivity to the level of reference population. This might be because transplant photosynthate was preferentially translocated to above-ground tissues during the initial period of transplantation. Thus, a longer time was needed for transplant below-ground productivity to reach the level of reference population than for above-ground productivity.

Transplants of different seagrass species require different times to establish in a transplant site (Fonseca et al., 1996; Paling et al., 2001b; Holbrook et al., 2002; Meehan and West, 2002; Campbell and Paling, 2003; van Keulen et al., 2003; Horn et al., 2009). *Zostera marina* transplants usually required less time for establishment than other seagrass species (Paling et al., 2001b, 2007; van Keulen et al., 2003; Park and Lee, 2007). The establishment time of seagrass transplants also varied considerably among different planting methods (Fonseca et al., 1996; Paling et al., 2001b; Holbrook et al., 2002; Meehan and West, 2002; Campbell and Paling, 2003; van Keulen et al., 2003; Horn et al., 2009; Table 1). Based on the shoot density of transplants, the time required for transplant establishment was 1.0–2.3 months for the staple method, 2.2–2.6 months for the TERFS method, and 3.2–3.7 months for the shell method in *Z. marina* (Park and Lee, 2007; Table 1). In the present study, transplants planted using the staple method required 5–6 months to establish in the planting area.

In conclusion, although the eelgrass transplants were significantly smaller than reference population during the initial transplantation period, they exhibited great morphological plasticity after transplantation. After 5–6 months, the transplants recovered from the initial transplanting stress and established a larger plant size in the transplanting area, comparable or even greater individual shoot weight, and productivity above that of reference population. Monitoring of shoot morphology and growth as well as shoot density and survival rates was very effective for more accurate assessment of the establishment success of seagrass transplants.

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