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Note

An effective transplanting technique using shells for restoration of *Zostera marina* habitats

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Abstract

Significant declines in seagrass coverage have occurred in many parts of the world. In recent decades, transplanting projects for seagrass restoration at die-off areas have been attempted, but most current seagrass transplanting techniques are cost and labor intensive. We have developed a new seagrass transplanting method in which oyster shells are used as an anchoring device, and does not require SCUBA diving for sub-tidal planting. Here, we tested the shell method for feasibility and efficiency in large-scale seagrass restoration. Planting units consisting of two *Zostera marina* shoots anchored to one oyster shell were dropped from a boat to settle on the sediment at the test site. Four transplanting trials were conducted throughout the experimental period (December 2003, January, February, and November 2004) in Koje Bay on the southern coast of Korea. Eelgrass shoots planted using the shell method successfully established at the test areas, and the survival rates of transplants were comparable to those obtained using other common planting techniques. The transplant shoot density declined during the first 2–3 months following transplantation due to the initial transplant shock, and then surviving shoots became established at the sites and produced new lateral shoots after these periods. Plant size and leaf productivity of transplants 7 months post-transplanting were similar to or exceeded those of pre-existed shoots, suggesting that the physiological status of transplants is similar to that of natural population after 7 months. Because the shell method did not require workers to be in the water, the method was cost and labor effective. Additionally, given that shells originate from marine environments, the shell method did not leave any hazardous materials in the transplanting areas after restoration.

Keywords: Eelgrass; Restoration; Shell method; Transplanting method; *Zostera marina*

1. Introduction

Natural and anthropogenic disturbances in the coastal and estuarine areas have resulted in declines in seagrass coverage worldwide (Short and Wyllie-Echeverria, 1996). Seagrass die-off tends to be rapid, whereas the natural recovery of disturbed seagrass habitats is comparatively slow (Preen et al., 1995; Kirkman, 1998; Meehan and West, 2000). Therefore, numerous restoration projects through transplanting seagrasses have been attempted worldwide (Thorhaug, 1987; West et al., 1990; Fonseca et al., 1994; Kirkman, 1998; Orth et al., 1999; Campbell and Paling, 2003; Fishman et al., 2004). Since survival of seagrass transplants can be affected by the environmental conditions of planting sites, a site-selection model has been developed to select optimal transplanting areas (Short et al., 2002). Seagrass transplanting techniques using either shoots (seedlings or mature plants) or seeds have been developed to restore damaged seagrass beds, as well as to create new ones (Addy, 1947; Phillips, 1974; Thorhaug, 1987; West et al., 1990; Fonseca et al., 1994, 1998; Davis and Short, 1997; Orth et al., 1999; van Katwijk and Hermus, 2000; Fishman et al., 2004). Seagrass seeds can be easily collected from mature reproductive shoots, and sowing seeds is an economically effective method for large-scale restoration (Orth et al., 1994, 2000; Harwell and Orth 1999). A buoy-deployed system has been developed for effective eelgrass seed dispersal (Pickerell et al., 2005). However, the seed-broadcast technique is only useful when

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the seeds can settle and germinate, such as in areas with low seed predation and little physical disturbance.

Thus far, transplantation techniques have focused on moving vegetative shoots from donor beds to restoration sites (Fonseca et al., 1994; Davis and Short, 1997; Orth et al., 1999; Paling et al., 2001). Several techniques for transplanting vegetative shoots have been shown to result in the successful establishment of seagrass populations, but most techniques are labor intensive and expensive (Fonseca et al., 1994; Davis and Short, 1997; Orth et al., 1999; Paling et al., 2001). For example, SCUBA diving is often required for transplanting in deep water. This significantly increases planting costs, especially in areas with poor visibility (Fonseca et al., 1994; Orth et al., 1999).

Vegetative shoot transplanting techniques include sediment-associated and sediment-free methods. Sediment-associated methods such as the plug/core or sod method can minimize disruption to root and rhizome tissues, resulting in successful transplant establishment (Fonseca et al., 1994; Paling et al., 2001). However, the disadvantage of this method is that it can cause physical disturbances in the healthy donor bed (Fonseca et al., 1994). In addition, this method requires much time for the collection and transport of seagrass plugs, and is more costly than sediment-free methods (Fonseca et al., 1994, 1996). Sediment-free methods involve removing seagrass shoots along with bare roots and rhizomes from donor beds. The shoots are then anchored using devices such as staples, nails, rods, and frame systems because of the positive buoyancy of seagrass shoots (Phillips, 1990; West et al., 1990; Davis and Short, 1997; Fonseca et al., 1998). The bare-root method is a reliable transplanting technique; however, it is still labor intensive and requires divers for sub-tidal transplanting (Davis and Short, 1997; Orth et al., 1999).

Most of the original large seagrass beds on the coast of Korea were located in the bay systems of urbanized areas and have disappeared as a result of eutrophication (Lee and Lee, 2003). Therefore, large-scale seagrass restoration efforts are needed to recover biodiversity and reduced coastal production. Here, we describe a cost- and labor-effective technique for large-scale eelgrass restoration that uses oyster shells as an anchoring device. This technique does not require SCUBA diving for sub-tidal transplanting and it eliminates the need to retrieve the anchoring device because oyster shells are of marine origin.

2. Methods

2.1. Site location and eelgrass collection

The transplanting site was located in Koje Bay on the southern coast of the Korean peninsula (35°46′N, 128°34′E; Fig. 1). This site originally had a healthy *Zostera marina* meadow (Lee et al., 2005), but eelgrass shoots disappeared from the study site during the seashore road construction in spring 2003. Eelgrass transplanting using

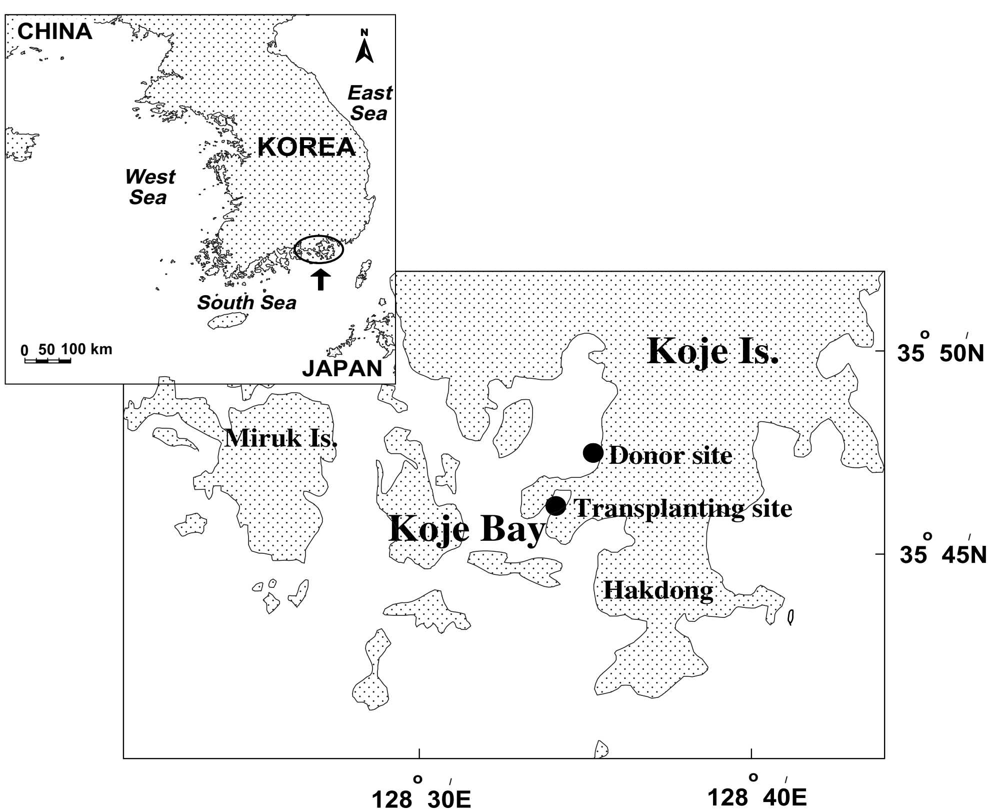

Fig. 1. Map indicating the plantation and donor sites in Koje Bay on the southern coast of the Korean peninsula.

the shell method was conducted in the bare area at an average water depth of 4 m relative to the mean sea level (MSL). Sediment at the site was characterized as silt loam.

Eelgrass shoots used for transplanting were collected from a large healthy donor bed in the vicinity of the transplanting site (Fig. 1). Shoots were collected individually by hand to minimize damage to the donor bed during low tide. Water depth at the donor bed during low tide was usually less than 50 cm. Each shoot had healthy leaf blades and five to seven rhizome/root nodes. The collected shoots were temporarily stored in large coolers with seawater to prevent desiccation and were transplanted within 24 h.

2.2. Transplanting method

A variety of types of clamshells can be used in this transplanting method; we used oyster shell >10 g in weight. Two holes with 5–7 mm diameter were drilled in each shell, and an eelgrass shoot was inserted into each hole from the rhizome, thus creating a single planting unit (Fig. 2A). The diameter of the holes was changed depend on the rhizome diameter of the donor shoots. The units were then stored in large containers with seawater and delivered to the planting site by boat. The shells cause the planting units to have negative buoyancy, thus allowing them to be placed on the sediment at the planting site by dropping them from the boat (Fig. 2B). Forty planting units were relocated in each 1-m^2 plot to achieve the experimental shoot density of 80 shoots m^{-2}, and four plots were planted at each planting trial. Four transplanting trials were conducted throughout the experimental period: December 2003, January, February, and November 2004.

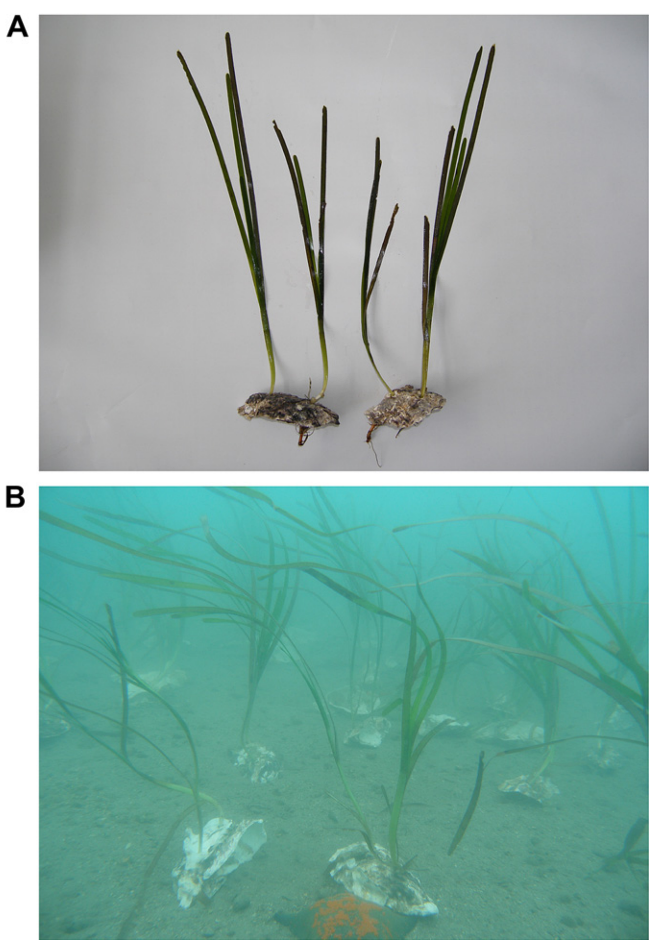

Fig. 2. (A) Planting unit used in the shell method, consisting of two eelgrass shoots and one oyster shell. (B) Planting units dropped from a boat onto the sediment of the transplanting site.

2.3. Monitoring transplants

To monitor the shoot density of the transplants, surviving transplants in the planting plots were counted monthly. Transplant survival rate was calculated as the percentage of plants that survived after the time required for the establishment of eelgrass transplants that occurred 2–3 months following transplantation. Eelgrass transplants showed high mortality during 2–3 months after transplantation due to the initial transplant shock. Eelgrass shoots, which could not take root at the planting sites by producing new below-ground tissues, disappeared during these periods. Survived transplants produced new lateral shoots, and thus shoot density increased after passing the period of the initial transplant shock. Therefore, survival transplants after the initial transplant shock were considered establishing at the transplanting sites. Leaf productivity, shoot height, and leaf width of eelgrass shoots transplanted in December 2003 were measured in April and July 2004, and January 2005 (4, 7, and 13 months post-transplanting, respectively). To assess the morphological and physiological status of the transplants, natural eelgrass shoots formerly existing at the transplanting area (i.e., in 2001–2003; Lee et al., 2005) were used as controls. Because these characteristics exhibit seasonal variation, transplants in April and July 2004 and January 2005 were compared with natural shoots in the same months, i.e., April, July, and January 2001–2003, respectively. Leaf productivity and shoot morphology of formerly existing natural shoots at the planting site during 2001–2003 adapted from Lee et al. (2005).

2.4. Statistics

All values are reported as mean ± standard error. Statistical analyses were performed using general linear models (SAS). Data were tested for normality and homogeneity of variance to meet the assumptions of parametric statistics, and assumptions were satisfied for all data tested. Differences in transplant density between sampling times and survival rates between the transplanting trials were examined using one-way ANOVA. A t-test was used to test for significant differences in leaf productivity and shoot morphology between transplants and natural eelgrass shoots.

3. Results

3.1. Shoot density and establishment of transplants

In all transplanting trials, the shoot density of eelgrass transplants declined during the first 2–3 months following transplantation due to the initial transplant shock (Fig. 3), after which transplants became established at the transplanting sites. The time required for the establishment of *Z. marina* transplants planted using the shell method ranged from 2.4 months for February 2004 planting trial to 3.4 months for November 2004 trial (Table 1). Survival rates after establishment were >75% and were not significantly different among the four transplanting trials ($P = 0.838$; Table 1). Transplant density exhibited significant ($P < 0.001$) seasonal variation, with the highest shoot density in summer and the lowest in late fall (Fig. 3). Transplant density reached approximately 130 shoots m^{-2} in the second summer following transplantation. Eelgrass shoots were not observed at the unplanted area around the planting plots throughout the experimental periods.

3.2. Transplant shoot morphology and leaf productivity

Shoot height, leaf width, and leaf productivity of the transplants showed significant temporal variation, with

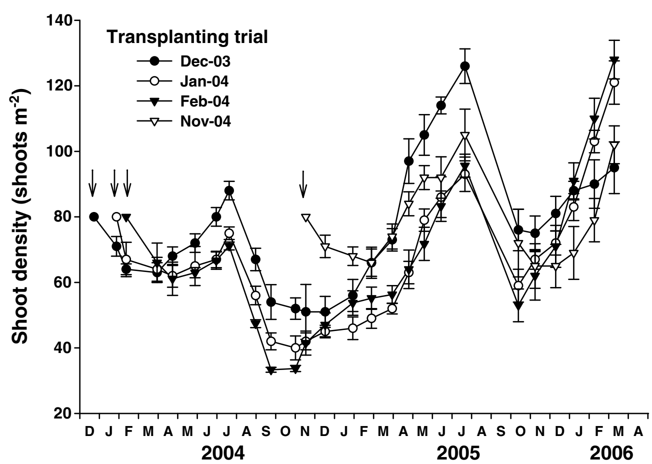

Fig. 3. Shoot density of eelgrass transplants since time of planting in December 2003, and January, February, and November 2004. Arrows indicate transplanting dates.

Table 1
The time required for the establishment of *Zostera marina* transplants planted using the shell method, and shoot density and percent survival (mean ± SE) after establishment at the transplanting site

Transplanting trial	Time for establishment (months since transplanting)	Transplant shoot density after establishment (shoots m^{-2})	Percent survival after establishment (%)
December 2003	3.3	63.0 ± 3.0	78.8 ± 3.8
January 2004	2.9	62.0 ± 3.5	77.5 ± 4.3
February 2004	2.4	61.1 ± 5.1	76.4 ± 6.4
November 2004	3.4	66.0 ± 4.2	82.5 ± 5.2

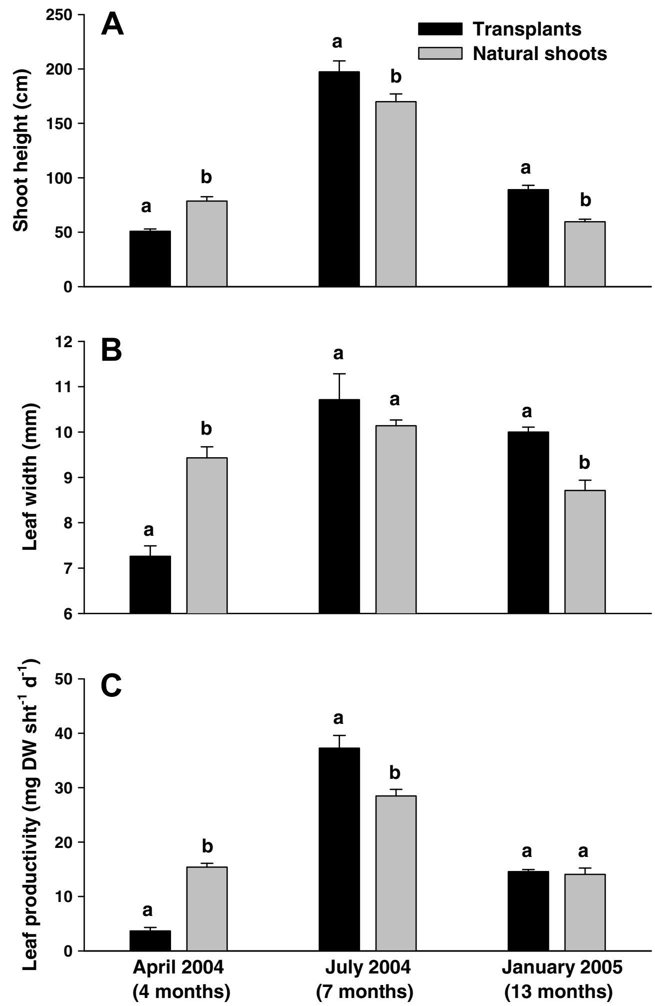

Fig. 4. (A) Shoot height, (B) leaf width, and (C) leaf productivity of eelgrass transplants planted in December 2003 and formerly existing natural shoots at the transplanting area. Shoot morphology and productivity of transplants 4, 7, and 13 months after transplanting were compared with those of formerly existing shoots in the same months. Leaf productivity and shoot morphology of formerly existing natural shoots at the planting site during 2001–2003 adapted from Lee et al. (2005).

the highest values in July, and the lowest in April and January ($P < 0.001$; Fig. 4). Eelgrass transplants were significantly shorter 4 months post-transplantation than formerly existing natural shoots (51 cm vs. 79 cm, respectively; $P < 0.001$; Fig. 4A), whereas, in July 2004 and January 2005 (7 and 13 months post-transplantation, respectively), transplants were significantly taller than natural control shoots.

Leaf width and productivity showed similar trends to shoot height. Leaves were significantly narrower on transplants 4 months after transplantation compared to those of formerly existing shoots at the same time of year (7.3 vs. 9.4 mm, respectively; $P < 0.001$), whereas the leaf width of transplants and natural shoots was not significantly different 7 months post-transplantation ($P = 0.79$; Fig. 4B). The leaf productivity of transplants 4 months after transplantation was also significantly lower than that of formerly existing shoots (3.7 vs. 15.4 mg dry weight shoot^{-1} d^{-1}, respectively; $P < 0.001$). Seven months after transplantation, the leaf productivity of transplants was significantly higher than that of control shoots at the same time of year ($P < 0.001$; Fig. 4C).

4. Discussion

4.1. Transplant survival rates

Z. marina transplants planted using the newly developed shell method established successfully in the test plots. Previous transplanting trials show that survival rates of seagrass transplants are highly variable depending on species and planting techniques (West et al., 1990; Fonseca et al., 1994; Davis and Short, 1997; Sheridan et al., 1998; Orth et al., 1999; Paling et al., 2001; Meehan and West, 2002; Campbell and Paling, 2003; van Keulen et al., 2003; Fishman et al., 2004). The mean survival rate of eelgrass transplants using the horizontal rhizome method, in which two shoots were secured horizontally into the sediment using a bamboo staple, was 71% (Davis and Short, 1997). Orth et al. (1999) reported an eelgrass transplant survival rate of 72.7% 1 month after transplantation using single unanchored shoots. Using the shell method, >75% of transplants survived after the transplant establishment at the planting sites that occurred 2–3 months following transplantation. This survival rate is comparable to those of other transplanting techniques (Table 2).

We saw an initial decline in transplants in the first 2–3 months following transplantation. An initial loss of seagrass transplants has been observed for most transplanting attempts and appears to be caused by disturbances such as storms, high wave energy, bioturbation, predation, and human activities that affect the survival of transplants before their establishment at the recipient site (van Tussenbroek, 1996; Davis et al., 1998; Campbell and Paling, 2003; Paling et al., 2003). After this initial loss, however, the transplant became established at the planting sites and the shoot density increased via lateral shoot production from the transplants.

Examining the morphological and physiological characteristics of transplants adapting to the new environment of the recipient site can help in developing criteria for defining successful seagrass restoration (Phillips and Lewis, 1983; Meinesz et al., 1993). Plant size and leaf productivity of transplants 4 months post-planting were lower than those of the shoots formerly existing in the same area, whereas after 7 months, the values were similar to or exceeded those of the control shoots. These results suggest that the physiological status of transplants at their new environment become to be similar to that of natural population after 7 months following transplantation.

After the establishment, shoot density showed seasonal patterns similar to those of the formerly existing natural bed. Transplant density significantly decreased by 30–50 shoots m^{-2} during the first fall after transplantation. Natural eelgrass populations along the coast of Korea

Table 2
Percent survival of seagrass transplants planted using previously developed methods and the shell method

Planting method	Species	Percent survival (range)	Monitoring time for % survival (days since transplanting)	Reference
Unanchored	*Zostera marina*	73	1 month	Orth et al. (1999)
Coring	*Halodule wrightii*	93	96 days	Fonseca et al. (1994)
	Syringodium filiforme	79	96 days	Fonseca et al. (1994)
Peat pot	*Halodule wrightii*	96	96 days	Fonseca et al. (1994)
	Syringodium filiforme	62	96 days	Fonseca et al. (1994)
	Halodule wrightii	56–84	3–4 months	Sheridan et al. (1998)
Planting boat	*Zostera marina*	40 (24, 56)	1 week	Fishman et al. (2004)
Plug	*Amphibolis griffithii*	60	1 year	van Keulen et al. (2003)
	Posidonia sinuosa	0	1 year	van Keulen et al. (2003)
Sod	*Amphibolis griffithii*	44	2 years	Paling et al. (2001)
	Posidonia coriacea	76	2 years	Paling et al. (2001)
	Posidonia sinuosa	77	2 years	Paling et al. (2001)
Horizontal rhizome	*Zostera marina*	71 (1–99)	1 year	Davis and Short (1997)
Staple	*Halodule wrightii*	89	96 days	Fonseca et al. (1994)
	Syringodium filiforme	85	96 days	Fonseca et al. (1994)
	Halodule wrightii	69 (55–93)	4 months	Fonseca et al. (1996)
Wire peg	*Posidonia australis*	80–90	3 months	West et al. (1990)
	Zostera capricorni	60–70		
Plug with artificial seagrass mat	*Posidonia australis*	49	18 months	Campbell and Paling (2003)
Metal mesh	*Posidonia australis*	0–300	16 months	Meehan and West (2002)
Shell	*Zostera marina*	78 (73–83)	2–3 months	This study

experience declines in shoot density in late summer because of high water temperatures and tend to exhibit minimum shoot density in the fall (Lee et al., 2005). Thus, decreased transplant shoot density during late summer and fall was likely a result of natural seasonal fluctuations rather than transplantation failure. During the second year following transplantation, shoot density at the test sites increased by approximately 150% through vegetative propagation, thereby reaching levels similar to the formerly existing bed which has been studied in 2001–2003 (Lee et al., 2005). Therefore, within 2 years of planting using the shell method, the transplant area had recovered to levels recorded before the destruction of the natural bed.

4.2. Effectiveness of the shell method

Because of the positive buoyancy of seagrass shoots, anchoring mechanisms are required to fix the shoots to the sediment for successful establishment to occur (Davis and Short, 1997; Fonseca et al., 1998). Various anchoring devices such as staples, wire mesh, nails, and TERFS (transplanting eelgrass remotely with frame systems) have been designed to develop effective transplanting techniques (reviewed in Fonseca et al., 1998). These techniques have proven to be labor intensive and expensive. In most cases, seagrass shoots need to be individually anchored by hand, and sub-tidal planting requires the use of divers. The TERFS method was developed to minimize the cost of diving (Fred Short, University of New Hampshire), but requires that the planting frames be retrieved after a suitable rooting time of 1–2 months. Orth et al. (1999) developed a simple transplant technique where single unanchored eelgrass shoots with rhizomes were planted into the sediment at an angle. This method was highly successful but labor intensive because the shoots needed to be inserted by hand, again requiring SCUBA equipment for sub-tidal planting.

In contrast, the shell method was both cost and labor effective. Transplant units with two eelgrass shoots and one oyster shell were prepared onshore and were dropped to the recipient sites from a boat. Therefore, this method did not require divers to anchor the eelgrass shoots to the sediment, thus reducing the cost and labor required for the restoration of damaged eelgrass beds. Additionally, eelgrass planting by the shell method was 3–5 times faster than planting by the traditional staple method. By the rough estimation, the shell method reduced the cost for the eelgrass transplantation by 50–70% compared to the traditional staple method.

Because this technique does not require workers to be in the water, the shell method is suitable for transplanting in contaminated areas that might otherwise be costly and hazardous to restore. In addition, given that oyster shells are of marine origin, this method does not leave any hazardous materials at the recipient sites after transplantation, thereby eliminating the need to recover the anchoring devices. In conclusion, eelgrass transplants planted using the shell method successfully established in the test sites, and the recipient areas recovered to levels recorded before their destruction in the second year following transplantation. The survival of transplants reached rates comparable to those obtained using previously developed transplanting techniques, but required fewer resources to do so. Therefore, we think that the shell method is an effective method for seagrass transplantation, and can be incorporated into seagrass restoration strategies worldwide.

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