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# Comparison of seasonal growth responses of *Zostera marina* transplants to determine the optimal transplant season for habitat restoration

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ABSTRACT

Significant losses in seagrass coverage have been reported worldwide, and thus efforts are under way to restore disturbed seagrass habitats. Unfortunately, rates of seagrass restoration success through transplantation remain quite low, and inappropriate transplant times may be one cause of the low success rates. To determine suitable seasons for transplanting eelgrass, transplantation experiments were conducted seasonally. After each transplantation trial, shoot density, chlorophyll content, shoot morphology, and productivity of transplants and reference plants were monitored at 2–4-week intervals for approximately 3 months. All eelgrass transplants had disappeared by the end of the summer transplantation trial, whereas transplant density increased most rapidly in the fall transplantation. High water temperature in summer appeared to be a primary seasonal stress in eelgrass meadows, causing high transplant mortality. Results for shoot morphology, physiology, and growth of transplants indicated that those planted in summer suffered the most severe transplanting stress, whereas those planted in fall experienced the least stress. Accordingly, summer transplantation for eelgrass restoration should be avoided in areas where summer water temperatures are greater than 25 °C. Because transplants planted in fall exhibited the highest expansion of shoots and suffered the least transplant stress, the optimal season for eelgrass transplantation is likely to be fall, just after the high water temperature period.



## 1. Introduction

Seagrasses cover about 0.1–0.2% of the global ocean and often develop into highly productive systems that play a key role in coastal ecosystems (Duarte, 2002). Seagrass beds have been recognized as valuable resources critical to the health and function of coastal waters. Seagrasses generally require an underwater irradiance in excess of 11% of incident levels at the water surface for growth, a requirement that typically sets their depth limit (Duarte, 1991). Because of their high light requirements relative to macroalgae and phytoplankton, seagrasses occur in shallow, nearshore waters, rendering them highly susceptible to damage by human activities (Ralph et al., 2007). High turbidity associated with sedimentation and eutrophication has been suggested as a main cause of seagrass declines (Cambridge et al., 1986; Giesen et al., 1990; Erftemeijer and Lewis, 2006; Orth et al., 2006; Ralph et al., 2006). In addition, increasing expanses of coastal areas are being occupied by human structures and activities, exacerbating the situation for seagrass beds (Short and Wyllie-Echeverria, 1996; Touchette, 2007; Waycott et al., 2009).

Concern about seagrass loss has led to increased focus by environmental agencies and regulatory authorities on activities that impact coastal environments worldwide (Baden et al., 2003; Orth et al., 2010). Measures have also been taken to mitigate the decline of seagrass meadows, including improvements in water quality, limiting coastal construction, and restoring degraded seagrass beds (Bos et al., 2005; Golden et al., 2010; Orth et al., 2010). A consensus generally holds that seagrass beds can be restored

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through human-assisted recolonization, particularly when the areas experience improvements in water quality and/or other physical and chemical disturbances causing seagrass decline are removed (van Katwijk et al., 2009). Compared to upland reforestation, seagrass restoration involves a much more recent technology and has received less attention, funding, and study, although considerable research has been conducted, and success has been achieved in many parts of the world (Davis and Short, 1997; van Katwijk and Hermus, 2000; Calumpong and Fonseca, 2001; Paling et al., 2001, 2009; Orth et al., 2009, 2012).

The success rate of seagrass transplantation and restoration has been reported as only around 30% worldwide (Orth et al., 2006). Environmental factors such as sediment type, nutrient availability, water dynamics, water quality, water depth, and season are important factors affecting transplant success (Dennison and Alberte, 1986; van Katwijk and Hermus, 2000; Short et al., 2002; Park and Lee, 2010; Hovey et al., 2011, 2012). Water temperature, which is an important factor controlling seagrass growth, may also play a crucial role in transplant success.

Eelgrass (*Zostera marina* L.) is widely distributed across the Northern Hemisphere (20°–70°N) in shallow coastal areas with sandy and muddy sediments, where it exhibits diverse life history traits, morphology, and growth dynamics (den Hartog, 1970; Green and Short, 2003; Hauxwell et al., 2006). Similar to most other seagrass species, eelgrass is also experiencing global declines, and this species is the most commonly used for habitat restoration (Moore and Short, 2006). As a temperate seagrass species, eelgrass populations usually exhibit clear seasonal variation in morphology, growth, and reproduction (Boström et al., 2004; Hauxwell et al., 2006; Moore and Short, 2006). Therefore, eelgrass transplant survival may also be affected by the season during which the transplant occurs (Zimmerman et al., 1995; Park and Lee, 2007; van Katwijk et al., 2009). Thus, determining the appropriate planting season can often be the decisive factor affecting restoration success (van Katwijk and Hermus, 2000; Park and Lee, 2007; Golden et al., 2010).

The period immediately following the highest seasonal stress has been suggested as the optimal transplant timing (Calumpong and Fonseca, 2001; Park and Lee, 2007). Significantly diminished *Z. marina* growth has been observed during high water temperature periods in summer (Orth and Moore, 1986; Lee et al., 2005; Moore and Jarvis, 2008; Moore et al., 2012, 2014). Thus, the period of time right after high water temperatures has been considered an optimal season for eelgrass transplantation (Park and Lee, 2007). Seagrass transplants may exhibit high mortality during the initial period of transplantation due to transplant stress, often resulting from injury, desiccation, and impaired function during the planting process (Zimmerman et al., 1995; Struve et al., 2000; Horn et al., 2009). After transplant establishment by root growth at the planting sites, seagrass transplants will often suffer seasonal stresses at the planting sites, which can also cause transplant mortality (Davis and Short, 1997; Park and Lee, 2007; Rodríguez-Salinas et al., 2010; Tanner et al., 2010). Although a few studies have suggested appropriate seasons for seagrass transplantation by monitoring transplant density or survival rate (Table 1), almost none have closely examined the optimal transplanting time using measurements of growth and physiological responses of transplants to transplant stress. In the present study, we transplanted eelgrass on a seasonal basis and compared growth and physiological responses of eelgrass transplants to the initial transplant stress to determine the optimal transplant season. Transplants were assumed to be established at the transplant site when the morphology and physiology of transplants reached levels similar to those of reference plants (natural, non-transplanted plants growing in a similar environment).

## 2. Materials and methods

### 2.1. Study site

The transplant site was located in Jindong Bay (35°05.6′N, 128°33.6′E) on the south coast of Korea (Fig. 1). Eelgrass was once widespread at this site, but most eelgrass meadows have disappeared due to seashore road construction, fishery activities, increased nutrient loading, and residential and commercial dredge-and-fill projects. Conditions within the water column and sediments of the bay have improved considerably due to recent efforts to improve water quality; as a result, a few patches of *Z. marina* have been observed again in this bay system. The transplant site contains loam sediment and was located in a subtidal area with a water depth of about 1.0 m below mean lower low water (MLLW). Water temperature was measured at the transplant site every 15 min during the experimental periods using Hobo data loggers (Onset Computer Corp., Bourne, MA, USA) encased in underwater housing.

### 2.2. Seagrass transplantation

Eelgrass vegetative shoots were harvested from patches of dense eelgrass shoots in the vicinity of the planting site via SCUBA diving. When collecting eelgrass shoots, special care was taken to avoid damage or loss of above- and belowground tissues. Shoots were collected individually by hand to minimize damage to the donor bed. Intact shoots with six nodes of rhizomes with roots were selected for transplantation. Collected eelgrass shoots were planted in an area with sediments and water depths similar to those of donor patches to ensure that the planting sites and donor patches shared similar environmental conditions. Eelgrass shoots in the donor patches were used as reference plants for analyzing the responses and establishment of eelgrass transplants after transplantation. Eelgrass transplantations were conducted seasonally in July, October, and December 2008, as well as March 2009. Eelgrass shoots were hand-planted while SCUBA diving using the staple method, which anchored plants with bent metal wires. During each transplant season, four 2.0 × 2.0-m transplantation plots were established, and 128 shoots were planted in each plot. Thus, the initial transplant density was 32 shoots $m^{-2}$ during all transplant seasons. A 0.5 × 0.5-m permanent quadrat was established in each transplantation plot to measure changes in transplant shoot density during each transplant season.

### 2.3. Biological measurements

After transplantation, transplants and reference plants were monitored routinely at intervals of 2–4 weeks for approximately 3 months. At each monitoring event, the number of transplants within each permanent quadrat was counted to estimate transplant density. Ten to 12 eelgrass shoots (2–3 shoots from each transplantation plot) were collected from the transplant site (outside the permanent quadrats) and the donor patches for measurements of shoot morphology and leaf chlorophyll content.

Plants sampled for shoot morphology and leaf chlorophyll content were washed carefully with tap water and thoroughly cleaned of epiphytes and sediments. The number of leaves per shoot was counted from the upper end of the sheath, and shoot height was measured from the meristem to the tip of the longest leaf. Leaf width was determined at the middle of the longest leaf to the nearest 0.1 mm. The diameters of rhizome internodes from the first to the sixth internode (counted from the meristem) were also measured to the nearest 0.1 mm. The middle portion of the second youngest leaf was used for measurements of