





RESEARCH ARTICLE

Seed- versus transplant-based eelgrass (*Zostera marina* L.) restoration success in a temperate marine lake

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Despite active seagrass restoration gaining traction as a tool to halt and reverse worldwide seagrass losses, overall success remains limited. Restoration strategies, through seeding or transplantation, face different environmental bottlenecks that limit success. Choosing the most appropriate strategy of the two for a specific location, however, is hampered by lack of direct practical comparisons between strategies within a single system. To investigate potential life stage dependent bottlenecks, we compared seed-based and transplant-based restoration of *Zostera marina* in the subtidal saltwater Lake Grevelingen. Our results demonstrate that seedling recruitment was negatively impacted by bioturbation from the lugworm *Arenicola marina* and sediment movement due to hydrodynamic exposure. Transplant-based restoration was clearly more successful but surprisingly best predicted by leaf gluing by the ragworm *Platynereis dumerilii*. This previously undescribed interaction caused seagrass leaves to clump and reduce effective photosynthetic surface and leaf movement. We suggest that the observed behavior of these worms may result from a lack of foodweb interactions, illustrating the importance of trophic control for seagrass restoration. Thus, in addition to recognizing life stage dependent environmental bottlenecks for restoration strategy selection, seagrass restoration may also require the active recovery of their associated food webs.

Key words: *Arenicola marina*, hydrodynamics, leaf gluing, *Platynereis dumerilii*, sediment working, seedling recruitment, seedling survival, transplant survival, *Zostera marina*

Implications for Practice

- Seagrass restoration studies implementing multiple restoration strategies are limited, but can improve restoration success through informed strategy selection.
- Life stage-specific bottlenecks call for location specific restoration strategies. Specifically, subtidal conditions with high wave exposure and lugworm densities favor transplants from a perennial clonal donor population, whereas relatively calm, or intertidal areas profit most from the scaling potential of seed-based donor populations.
- Managers should be aware of potential negative interspecific interactions between seagrass and the microbenthic community caused by shifts in the species compositions, adding bottlenecks to restoration.

Introduction

Seagrasses are vascular rhizomatous plants that have conquered coastal and estuarine environments. Their meadows are highly valued for their ecological and economic functions, including carbon storage, nutrient cycling, nursery habitat for fishes, and

biodiversity enhancement (Nordlund et al. 2016; Unsworth et al. 2019). However, similar to other coastal ecosystems such as coral reefs, mangroves, and salt marshes, seagrass meadows have declined at an alarming pace over the past century, with a worldwide annual loss rate of 7% per year since 1990 (Waycott et al. 2009). This decline can primarily be attributed

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to anthropogenically driven factors such as eutrophication, infrastructure development, and poor management-based salinity shifts (Duarte 2002). To halt and reverse these worldwide losses, substantial efforts have been made to improve environmental conditions (Sherwood et al. 2017; de los Santos et al. 2019). In addition, conservation practitioners and policy makers are increasingly focusing on active habitat restoration as a potential part of conservation strategies to counter seagrass declines (Cunha et al. 2012; Eriander et al. 2016a; Orth et al. 2020).

Although seagrass restoration is gaining traction as a conservation tool, its success rate has thus far been limited compared to other marine restoration projects (Bayraktarov et al. 2015). In fact, a global analysis on seagrass restoration by van Katwijk et al. (2016) demonstrated that 37% of trials survived after 36 months, over a total of 1786 restoration trials. Multiple causes have been advanced to explain the observed high failure probability. First, general abiotic conditions may simply be insufficient when restoration is attempted (van der Heide et al. 2007). Second, extreme events (e.g. storms, siltation or salinity pulses, drought), relatively common to coastal ecosystems, can easily destroy restoration attempts (Orth et al. 2006; Paulo et al. 2019). Third, trophic interactions may also be disrupted, hampering recovery attempts. For instance, absence of top predators can lead to high numbers of meso-predators, that in turn overexploit invertebrate grazers required to control epiphytic algae growing on seagrass leaves (Hughes et al. 2013; Östman et al. 2016). Finally, nontrophic interactions, and particularly positive density-dependent facilitation are important factors for seagrass meadow stability that can be compromised by disturbance (Maxwell et al. 2017; Paulo et al. 2019), and most restoration trials are not of a sufficiently large scale to eventually overcome this (van Katwijk et al. 2016). To anticipate these obstacles, habitat suitability assessments are often the first step in restoration strategies (Short et al. 2002; van der Heide et al. 2009; Folmer et al. 2016), while stochasticity-related risks are often mitigated by simultaneously deploying restoration activities at multiple sites over multiple years (van Katwijk et al. 2009; Suykerbuyk et al. 2016).

Although restoration approaches vary per project, they are mainly based on the use of either seeds or transplants (van Katwijk et al. 2016). Both strategies offer advantages and disadvantages with seeds providing easier scaling opportunities and less damage to donor populations (Orth et al. 2020), while transplants need to overcome less bottlenecks. Each life stage copes with distinct bottlenecks; At their most early stage, seeds can be buried by bioturbation (Greve et al. 2005; Valdemarsen et al. 2011), transported to unsuitable areas via currents (Infantes et al. 2016), predated upon by the shore crab *Carcinus maenas* (Fishman & Orth 1996; Infantes et al. 2016), and affected by disease (Govers et al. 2016). Once germinated, small seedlings are still prone to burial by bioturbation (Philippart 1994; Valdemarsen et al. 2011; Suykerbuyk et al. 2016) and disease (Govers et al. 2017), but also to sediment and water dynamics causing dislodgement: seedlings are lighter than seeds and have smaller roots than adult plants. Like adult plants (Davis et al. 1998), seedlings may experience severe disturbance by *C. maenas*, as well as epiphyte overgrowth and

suffocation by macroalgae (Davis et al. 1998; Moksnes et al. 2018). Hence, the choice for either seeds or adult transplants may often depend strongly on the combination of both availability of source material and locally prevailing conditions. Yet, despite the importance of this choice for eventual restoration success, experimental data comparing restoration success of seeds versus adult transplants simultaneously at the same locations is currently limited (Eriander et al. 2016b).

In this study, we experimentally investigated potential bottlenecks for the success of both seed and transplant-based restoration of the seagrass *Zostera marina* L. (eelgrass) in the subtidal Lake Grevelingen—the largest saltwater lake in Western Europe—with the aim of selecting the best performing restoration strategy. More specifically, we tested the following hypotheses: (1) seedling recruitment and survival will be higher in areas with low sediment dynamics through hydrodynamic forcing and bioturbation, whereas adult transplant survival will be higher in more dynamic conditions, (2) cutting and predation by the *C. maenas* as well as suffocation by drifting algal mats will decrease recruitment success, seedling survival, and adult plant survival. Finally, we added a third hypothesis: gluing of seagrass leaves by the ragworm *Platynereis dumerilii* reduces survivorship of adult plants. We added this last hypothesis because during the experiments, we observed that *Z. marina* leaves were frequently glued by the ragworm *P. dumerilii* (Fig. 1D), which is known to glue macroalgal fragments together with mucus for the construction of tubes that reduce predation risk (Bedford & Moore 1984). *Platynereis dumerilii* tubes were also observed to be covered by plumes of epiphytes which they collect and attach to their tube for later consumption, a process called algal gardening (Woodin 1977; Fig. 1E). Although *P. dumerilii* is known to live in seagrass meadows (Daly 1973; Jacobs & Pierson 1979; Gambi et al. 2000), the gluing of seagrass leaves was previously undescribed and could potentially decrease restoration success. To test our hypotheses, we conducted two field experiments in which we introduced *Z. marina* through seeding and transplantation, respectively, at multiple subtidal sites, after which we monitored plant performance and potential limiting environmental factors.

Methods

Study Area

Our study was conducted at Lake Grevelingen, situated in the Province of Zeeland, the Netherlands (Fig. 1A). Covering about 10,800 ha, the lake was formerly an estuary with intertidal flats covered with low densities of *Z. marina* intersected by deep channels. In 1964, the estuary was transformed into an artificial saltwater lake by damming off the connections to both fresh water and seawater, as a protective measurement after a catastrophic flood in 1953. Nowadays, there are no tides and water level variation is negligible (5 cm). Salinity varies slightly between 27.7 and 31.6 ppt throughout the lake due to a small opening in the Brouwers Dam which separates the lake from the North Sea. Currents in the Lake are largely wind driven but can still reach high velocities, mainly during storms.

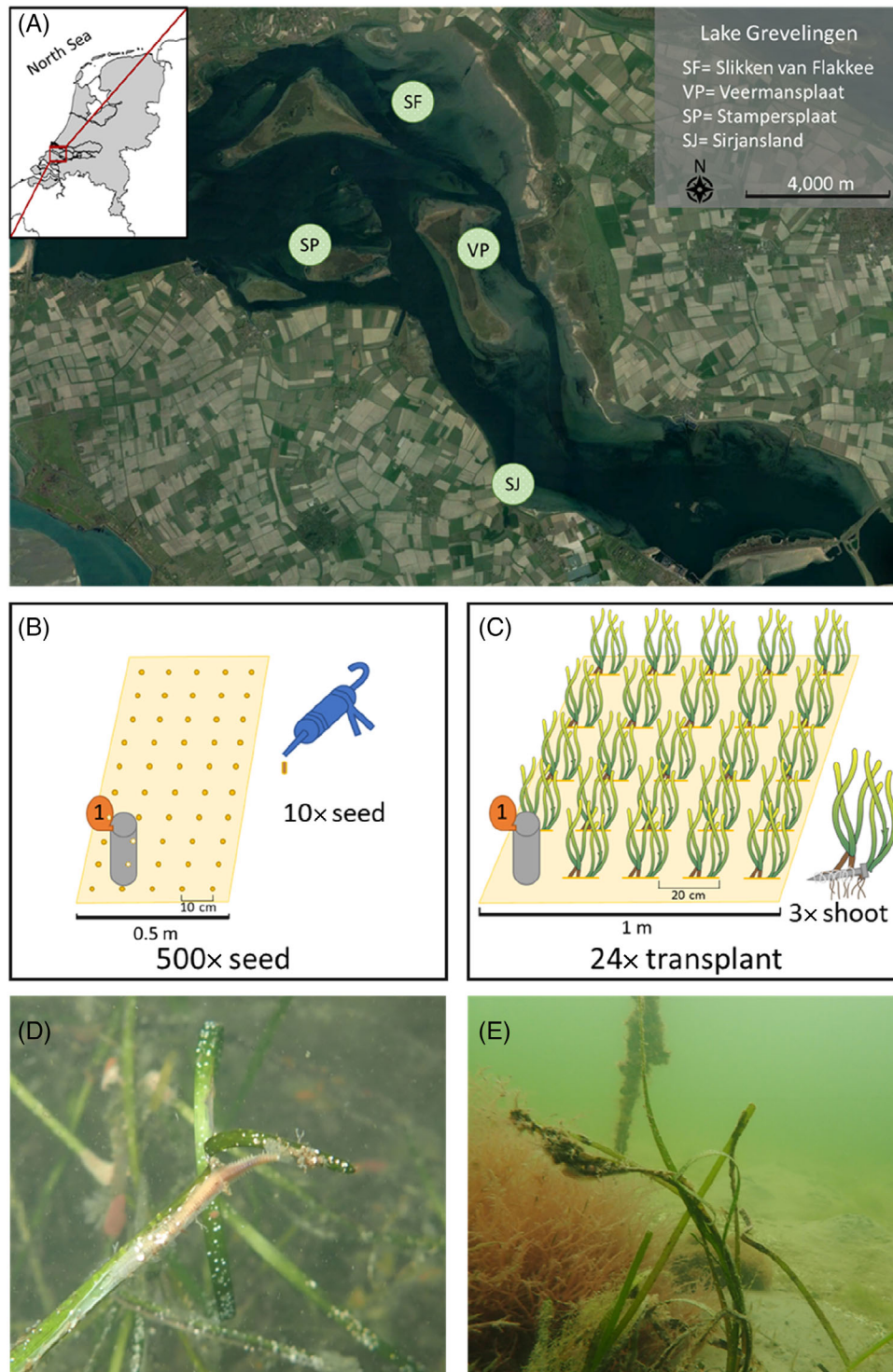


Figure 1. (A) Study area and field sites. Schematic illustration of the plot setup depicting a marked 0.5-m² plot containing (B) 500 seeds as well as a sealant gun injecting a sediment inject containing 10 seeds, and (C) a marked 1-m² plot containing 24 transplants as well as an example transplant unit with three shoots bound to a nail; (D) the ragworm *Platynereis dumerilii* within its tube just after removing glued leaves and algae; (E) *Zostera marina* transplant with leaves glued together by *P. dumerilii*, showing excessive epiphyte growth.

Shortly after the conversion to a lake, *Z. marina* started to dominate the former tidal flats coinciding with a salinity drop, covering a maximum area of 4,600 ha in 1978 (Nienhuis

et al. 1996). However, *Z. marina* disappeared in the late 1990s and remains absent to date. This rapid disappearance coincided with the reopening the connection with the North Sea, causing

an increase in salinity that may have contributed to the decline (Nienhuis et al. 1996). Afterwards, ephemeral macroalgae became the main primary producers. These short-lived algae show high spatial and temporal variability compared to sessile perennial seagrass plants and have yearly bloom and die off events. Also, several species that are known for their potential to inhibit seagrass restoration—larger individuals of the lugworm *Arenicola marina*, ragworms, and the shore crab *C. maenas*—are common in the lake (Mulder et al. 2019). Over the last decades however, conditions in Lake Grevelingen have stabilized and abiotic conditions seem favorable for seagrass restoration (van der Heide et al. 2009). Furthermore, interest in seagrass restoration in the lake has increased to further improve habitat quality. At present, *Z. marina* is considered a threatened species in the Netherlands. Its restoration in areas such as Lake Grevelingen will thus improve flora and is expected to indirectly improve faunal composition also. For successful restoration and well-informed management, experimentally uncovering potential bottlenecks as well as the most favorable restoration strategy is very valuable.

To test both seed- and transplant-based restoration strategies and select the best possible strategy for Lake Grevelingen, we conducted two separate restoration experiments with seeds and transplants respectively at four sites within the lake (Fig. 1A): (1) Slikken van Flakkee (SF: 51°47'4.8048"N, 3°58'28.8552"E), (2) Veermansplaat (VP: 51°44'55.6578"N, 4°0'10.548"E), (3) Stampersplaat (SP: 51°44'50.661"N, 3°56'8.4078"E), and (4) Sirjansland (SJ: 51°41'19.2228"N, 4°1'6.2358"E). The selected sites have a depth range of 90–130 cm, similar to the depth range of past peak *Z. marina* occurrences in Lake Grevelingen (Nienhuis et al. 1996). These sites were selected because they all share a history of *Z. marina* presence and consist of vast subtidal flats offering high potential for seagrass propagation once restoration has proven successful. Also, the sites differed considerably in environmental conditions (hydrodynamic exposure, lugworm, crab, and algae densities), allowing us to draw more generally valid conclusions for the lake as a whole with regard to seagrass restoration potential for each methodological approach. See Table S1 for a complete overview of environmental conditions per study site.

Donor Populations

Seeds and transplants were collected from two different donor populations. Both donor sites were in relative proximity (i.e. <1 day of transport) to Lake Grevelingen, limiting stress to plants and seeds during shipment. Seeds were collected in the German Wadden Sea at Hamburger Hallig (54°35'53.9"N, 8°48'40.8"E), an intertidal seagrass meadow with *Z. marina* plants that have a seed-based annual reproduction strategy, resulting in high seed production. Natural seed production and ripening in the meadow occurs during late summer and early fall; natural germination occurs in early spring. Transplants were collected at Denmark, Limfjord at a depth of ±80 cm (57°0'57.69736"N, 9°29'23.0172"E). This seagrass meadow with perennial *Z. marina* plants that predominately reproduce

vegetatively was selected for its density of seagrass shoots, vegetative reproduction, and similar growing conditions to Lake Grevelingen regarding average depth, limited tidal amplitude, salinity, and hydrodynamic exposure. Although growing conditions in Lake Grevelingen are most comparable to the Limfjord, the Wadden Sea population is more genetically similar to the original Lake Grevelingen population (Olsen et al. 2004). Note that the original Lake Grevelingen population also started from an intertidal, annual population similar to the German seed donor site (Beefink 1965).

Seedling Experiment

Zostera marina seeds were harvested in the late summer of 2018. Over the course of fall and winter, seeds were stored at 4–7°C in layers of max 1 cm with a continuous flow of artificial seawater (30 ppt, Tropic Marin) containing 0.2–0.5 ppm of copper sulfate as a treatment against *Phytophthora* infection (Govers et al. 2017). To stimulate simultaneous germination, seeds were placed in fresh water (Xu et al. 2016) for an hour prior to introduction in Lake Grevelingen. Next, seeds were mixed with mud, after which the resulting sludge/seed mixture was inserted into sealant guns following Tan et al. (2020). Using these sealant guns, a total of 10,000 seeds were injected 2 cm into the sediment in April 2019. At all 4 sites, 5 plots were injected with 500 seeds 0.5 m⁻² at a depth between 90 and 130 cm. Injections were evenly distributed by using a 0.5 m⁻² quadrat with a 10 × 10-cm grid, resulting in 50 injects with ±10 seeds per inject in each plot (Fig. 1B).

Transplantation Experiment

Zostera marina plants were harvested in June 2019. Transplants were removed by hand to minimize damage, after which they were stored in crates, and covered by wet cloths to retain moisture during transport. After transport, plants were stored in aerated basins filled with artificial sea water (29 ppt, Tropic Marin). Vegetative shoots with intact healthy rhizome and roots with at least three healthy leaves were selected for transplantation. Transplant units were created by bundling three vegetative shoots using iron wire, which were then attached to a 15-cm iron nail (according to Tan et al. 2020). Two days after harvest, 480 transplant units (1,440 shoots) were evenly distributed over five plots of 1 m² at each of the four sites (Fig. S1) which varied in depth between 90 and 130 cm. Per plot, 24 transplant units (72 shoots) were buried by hand in 4 cm deep gullies, were spaced 20 cm apart (Fig. 1C).

Field Measurements: Seagrass

The field sites were monitored weekly after seeding (22 April 2019), and monthly after the transplants were transplanted (14 June 2019) until August and October for the seed and transplant experiment, respectively. The following year, in August and November 2020, two additional checks were performed for the transplant experiment. Monitoring was done by snorkeling to get a close view without disturbing the sediment. During

monitoring, seedlings were counted individually. Our frequent monitoring after seed injection made it possible to distinguish two phases in the seedling experiment: (1) seedling recruitment success at the peak in recruitment when most germinated seedlings are present (late May, 29 days after the seed injection) resulting from germination success and losses during the germination period, and (2) the phase where germination had halted, and seedling survival was calculated in loss rates (%/day) from the number of seedlings compared to the highest number of seedlings present 29 days after injection. Since lateral expansion for *Z. marina* transplants is limited to on average 16 cm/year within established patches in Limfjorden, the donor field (Olesen & Sandjensen 1994), survival of transplants units per square meter was counted instead of shoots. During monitoring in the following year, shoot estimations were based on shoot counts within 0.25-m² quadrants, extrapolated to the seagrass patch surface area.

Field Measurements: Biotic Stressors

Field measurements for the environmental variables concerning seedling recruitment were done three times from early until late May 2019 at the seedling recruitment peak, 29 days after seeding (Table S1). Environmental variables for seedling survival were measured twice in June and July, respectively, after which all seedlings had disappeared. Environmental variables for the transplant experiment were measured monthly from June to October 2019. During each of these observation rounds, macroalgal cover (%) was estimated based on a 10 × 10-cm mazed 1-m² grid placed on top of the plots. After carefully removing any macroalgae that hamper sight, seedlings, transplants, *C. maenas*, and lugworm mounds were counted. Lugworm fecal mounds were used as a proxy of lugworm numbers (cf. Suykerbuyk et al. 2016). We counted mounds >4 cm diameter as an indicator of sediment reworking by larger lugworms, which have been shown to bury seeds beneath a critical depth (Valdemarsen et al. 2011). At the end of the growing season in late August, 76 days after transplantation, we discovered leave gluing by the ragworm *P. dumerilii* and counted the number of transplant units with leaves glued to calculate the percentage of still present glued transplants. Glued leaves could be easily recognized as clumps of two or more leaves that were attached to each other, often overgrown by epiphytes.

Field Measurements: Sediment Dynamics

Sediment displacement was used as a proxy for hydrodynamic forcing. At each plot a 1-m stainless-steel rod was firmly anchored to a depth of 80 cm. Next, a washer was placed around the rod on top of the sandy sediment surface as in Temmink et al. (2020). At each sampling instance, rod height above the sediment (r) and washer depth underneath the sediment (w) were measured, respectively, to determine sediment accumulation and erosion. Both were measured using a ruler to a precision of 0.5 cm. After measuring washer depth, the ring was placed

on top of the sediment again after which the new rod height above sediment was measured again (r^2). Sediment displacement (d) of time (t) was estimated using the following equation: $d_t = r_t - r_{t-1}^2 + 2 \times w_t$.

Data Analysis

For both the seed- and transplant-based experiment, we first analyzed differences between the four restoration sites within the lake, based on the peak in seedling recruitment (29 days after seeding) and transplant survival at the end of the growing season (76 days after transplantation). For this purpose, we used general linear models with site as fixed factor and the plots within each site as replicates, followed by Tukey's honestly significant difference post hoc tests to detect differences between individual sites.

As a second step, we explored to what extent single environmental variables measured at plot level could explain observed differences between plots within both experiments. For this purpose, we constructed separate generalized linear models per measured environmental variable. Specifically, for seedling recruitment, we constructed single-factor models including either lugworm density, sediment displacement, algae cover, or shore crab density as explanatory variables. For transplant survival, we constructed models including either sediment displacement, algae cover, shore crab density, or percentage still present transplants glued by *P. dumerilii*.

As a third step, we examined what combination of observed environmental factors could best explain our results. To this end, we combined all measured environmental factors into one model for both seedling recruitment and transplant survival experiments, respectively. We then applied stepwise backward removal of variables to select for the simplest best fitting model by using a minimal drop of 2 points in the model's AIC (Burnham & Anderson 2002). Furthermore, as part of the third step, we applied the same stepwise backward removal of variables for full models containing the mean of all explanatory variables up to the peak in seedling recruitment for seed plots and up to the end of the growing season for transplant plots.

Finally, to compare the relative success of seedlings versus transplants, loss rate of seedlings and transplants (in % loss/day) bridging field visits following peak recruitment were analyzed using a Welch's t test after testing for normality within groups. As a second test, we performed a Fisher's exact test to compare the long-term survival of seedling and transplant plots surviving the winter period into 2020, using binomial distribution.

Explanatory variables were log +1 transformed if this improved normality as was tested with a Shapiro-Wilk's test of normality. All tests were considered significant and reported at an alpha level of $p \leq 0.05$. Table S2 displays all found significant correlations. Alpha levels of $p > 0.05$ were considered nonsignificant and only reported as such. Means in text and figures are followed by the standard error of the mean (SEM). All analyses were conducted with R, version 4.1.0 (R Core Team 2021).

Results

Seedling Recruitment Success

Seedling recruitment success during its peak varied significantly across sites ($\chi^2 = 29.67$, $p < 0.001$; Fig. 2) with an overall mean of 3.15 ± 1.04 (SEM) seedlings per plot ($0.63 \pm 0.20\%$ of total number of injected seeds). None of the seeds successfully recruited at Sirjansland (SJ). At Slikken van Flakkee (SF), Veermansplaat (VP), and Stampersplaat (SP) seedling recruitment amounted $0.28 \pm 0.05\%$, $0.96 \pm 0.09\%$, and $1.28 \pm 0.32\%$, respectively, with significant difference between the best (SP) and the worst (SF) performing site of these three (Fig. 2).

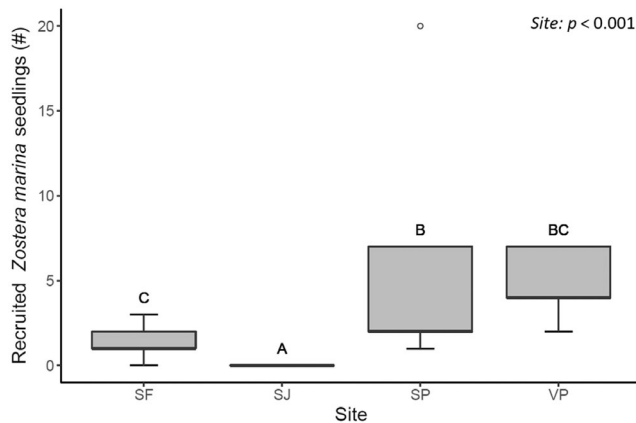


Figure 2. Boxplot representing recruited number of seedlings (number of counted seedlings per plot, 29 days after introduction for each study site: Sirjansland (SJ), Veermansplaat (VP), Slikken van Flakkee (SF), and Stampersplaat (SP). Different letters represent significant differences between groups derived from a Tukey's post hoc test.

When we explored how single environmental variables could explain observed differences, we found that seedling recruitment success correlated negatively with lugworm (mound size >4 cm diameter) density ($\chi = 14.77$, $p < 0.001$) and sediment displacement ($\chi = 7.17$, $p = 0.007$), respectively. Algae cover and shore crab density did not show a significant relationship with seedling recruitment success. When selecting for the best full model explaining seedling recruitment success by stepwise removal of environmental variables, lugworm density ($\chi = 27.87$, $p < 0.001$; Fig. 3A) and sediment displacement ($\chi = 11.47$, $p < 0.001$; Fig. 3B) remained, yielding an r^2 of 0.56 for the combined model. This outcome was the same when the full model was tested on the means of environmental variables up to the peak in recruitment (Table S1). After the recruitment peak, seedling numbers started to decline. With a mean daily seedling loss rate of 1.1% (per day) following the peak in seedling recruitment, the last remaining individuals disappeared 93 days after injection.

Transplant Survival

At the end of the growing season, 76 days after transplantation, the percentage of surviving transplants varied significantly across sites ($\chi = 17.89$, $p < 0.001$; Fig. 4). SF harbored the highest percentage of surviving transplants with $77.5 \pm 6.4\%$ of the initial planted transplants remaining. Sites SF and VP both harbored a significantly higher percentage surviving transplants than SP where only $38.3 \pm 7.4\%$ transplants remained.

When analyzing single environmental variables, transplant count showed a significant negative relationship with percentage glued transplants by *P. dumerilii* ($\chi = 11.09$, $p < 0.001$; Fig. 5A). When selecting for the best full model explaining transplant survival by stepwise removal of variables, again, only the percentage of glued transplants remained significant,

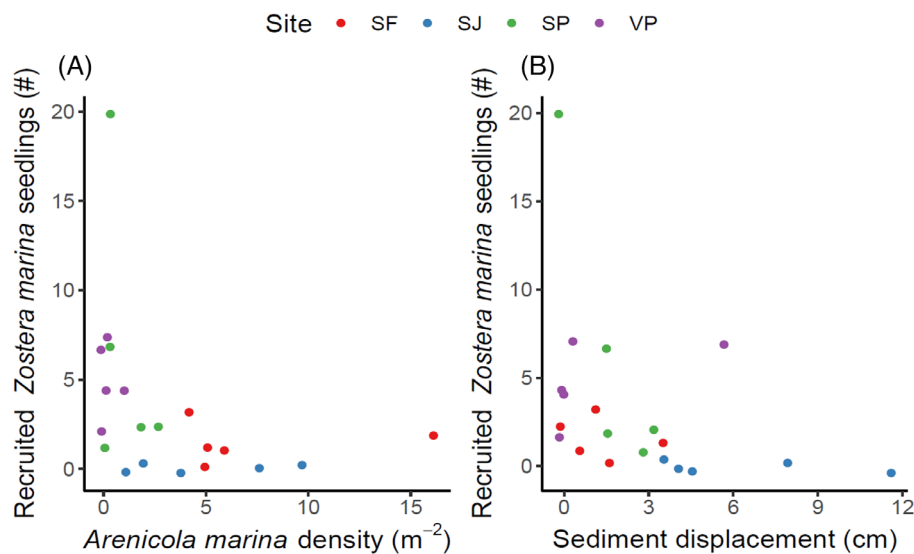


Figure 3. Scatter plots representing the correlation between *Zostera marina* recruited number of seedlings and (A) *Arenicola marina* (mound >4 cm diameter) density as well as (B) sediment displacement in centimeters 29 days after introduction ($n = 20$). Different colors represent the different study sites.

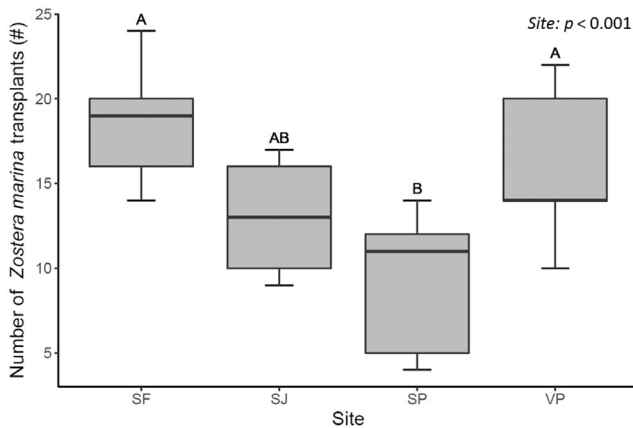


Figure 4. Boxplot representing *Zostera marina* transplant number per plot, 76 days following transplantation (27 August 2019) for each study site; Sirjansland (SJ), Veermansplaat (VP), Slikken van Flakkee (SF), and Stampersplaat (SP). One transplant unit comprises of three shoots. Different letters represent significant differences between groups derived from a Tukey's post hoc test.

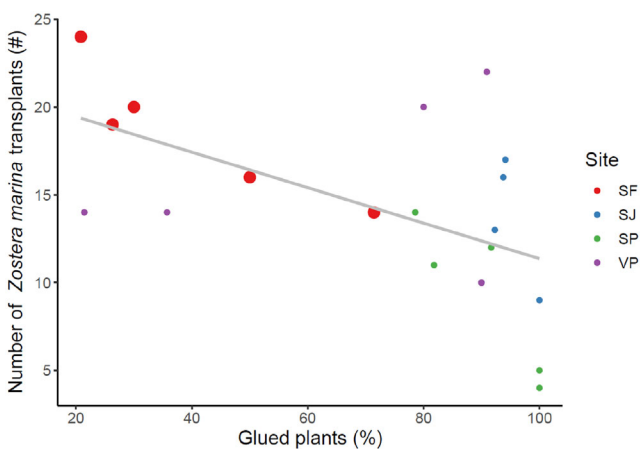


Figure 5. Correlation between *Zostera marina* transplant number and percentage of glued transplants, 76 days after transplantation ($n = 20$). One transplant unit comprises of three shoots. Different colors represent the different study sites. Large dots represent plots that survived into 2020.

yielding an r^2 of 0.43. This result remained the same when the full model was tested for the means of environmental variables up to the end of the growing season (Table S1). No relationship between plant survival and lugworm densities, sediment displacement, algae cover, or *Carcinus maenas* density could be found.

Comparing Seedlings to Plants

Loss rate (% loss per day) of seedlings after the initial recruitment peak was significantly higher than the loss rate of transplants (Welch two-sample t test; $t = 5.16$, $p = 0.014$). In fact, mean loss rate per day for transplants remained lower compared to seedlings during all monitoring rounds in which both seedling

and transplant losses were measured. No seedling survived until winter compared to the 220 transplants. During the following year, transplant-based seagrass plots locally survived at 5 of the initial 20 plots (Fig. 5) and expanded at 4, showing significantly higher survival compared to the 0 out of 20 surviving seed-based seagrass plots (Fisher's exact test; $p = 0.047$).

Discussion

Our experimental study on identifying life stage-specific bottlenecks hampering the success of seed- versus transplant-based restoration in subtidal conditions revealed that the lugworm *Arenicola marina* and sediment mobility as a proxy of hydrodynamic exposure negatively correlated with seedling recruitment. After recruitment, seedling numbers declined vast, resulting in 100% loss within 3 months. Transplant survival was much higher, with plants surviving in all plots until the end of growing season, and 25% of the plots surviving the winter and expanding the following year. Surprisingly, we discovered a clear negative relation with gluing by *P. dumerilii*, being the most important predictor for transplant survival. Overall, our results clearly demonstrate the importance of recognizing life-stage-dependent environmental bottlenecks for restoration strategy selection, and that temporary exclusion of negative species interactions (i.e. *P. dumerilii* for transplants and lugworms for seedlings) may be required to enhance restoration success.

Life Stage-Specific Bottlenecks: Recruitment Success and Survival

During the peak in seedling recruitment, 29 days after injection, we observed an overall low recruitment success ($0.67 \pm 0.18\%$), which is in accordance with *Z. marina* germination literature (Harrison 1993; Orth et al. 2003). Recruitment success was highly site specific (sites were separated by 5–10 km within the same lake) which is also line with previous findings (van Katwijk et al. 2016). This variability in seedling recruitment success negatively correlated with large (>4 cm fecal mound diameter) lugworms and sediment displacement through hydrodynamic forcing. Burial of seeds by lugworms below a critical depth of 5–6 cm prevents successful germination (Greve et al. 2005; Valdemarsen et al. 2011; Jarvis & Moore 2015). Where Valdemarsen et al. (2011) suggested that low densities of 5–10 lugworms/m² could negatively impact recruitment, we observed an effect at even lower densities when specifically considering large individuals. This can be explained by the fact that sediment reworking increases with size (Valdemarsen et al. 2011). Additionally, strong hydrodynamic forcing can prevent seedling recruitment via different pathways such as wave-driven horizontal seed dispersal (Delefosse & Kristensen 2012), physical stress (Valdemarsen et al. 2010), or exposure to predation by *C. maenas* (Fishman & Orth 1996; Infantes et al. 2016). Although we injected seeds at 2 cm depth, we found that 60% of the plots experienced hydrodynamics-related sediment displacement to depths beyond 2 cm prior to peak recruitment. Hence, seeds in these plots were likely dispersed and potentially vulnerable to the above mentioned

stressors, although we found no correlation between *C. maenas* density and recruitment success. Similarly, no correlation with algae cover was observed, most likely due to the low algae coverage during monitoring rounds.

Although low seedling survival (following recruitment) is encountered more often (Greve et al. 2005; Marion & Orth 2010; Valdemarsen et al. 2010), we could not link it to any, at plot level measured, environmental factors. However, we observed that the limited number of recruited seedlings experienced a steep decline after a storm hit one of the sites. Given our findings, we would need to dramatically increase seeding, or directly plant seedlings to overcome stochastic events at our study site. Overall, we find that the environmental settings in Lake Grevelingen do not favor seed-based restoration.

Life Stage-Specific Bottlenecks: Survival of Adult Plants

We found large variation between sites for transplant survival. Different from seed-based bottlenecks, transplant survival showed a significant negative relation with gluing by the ragworm *P. dumerilii*. Although *P. dumerilii* is common in seagrass systems (Daly 1973; Jacobs & Pierson 1979; Thormar et al. 2016), its gluing behavior was previously only described in macroalgae where the ragworm connects algae thalli by construction mucous tubes (Bedford & Moore 1984). By gluing multiple leaves with its dwelling tubes, plants became clumped, limiting the effective photosynthetic surface of the leaves as well as their mobility in the water column. Furthermore, *P. dumerilii* covers their tubes with epiphytes and algae for later consumption, a process called algal gardening, which further limits effective photosynthetic surface (Gambi et al. 2000). The fact that the observed gluing behavior was not described for seagrass meadows in literature before suggests that it has been overlooked thus far or is strongly controlled via ecological interactions in well-established seagrass systems. Gluing in established mature seagrass beds could for example be less obvious because mesograzers control epiphyte loads (Schanz et al. 2002; Hughes et al. 2004; Valentine & Duffy 2007). Grazers, often gastropods (Moksnes et al. 2008; Hughes et al. 2013) such as *Littorina* and *Hydrobia* spp., can be found in densities ranging from tens to thousands per square meters in seagrass beds (Thormar et al. 2016; Mulder et al. 2019). They can, however, decline dramatically in coastal waters due to trophic cascades (Moksnes et al. 2008; Baden et al. 2010; Hughes et al. 2013). Such a change in the food web might also have occurred in Lake Grevelingen. Although common three decades ago (Mulder et al. 2019), *Littorina* and *Hydrobia* spp. have largely disappeared in Lake Grevelingen (Schaub et al. 2002) and were not encountered at the sites where gluing was observed. However, to understand the true impact of gluing on seagrass survival, in our study system and its potential control by grazers in other seagrass systems, a controlled mesocosm experiment with crossed treatments of *P. dumerilii* and grazer additions should be a next step.

While none of the other environmental variables were found to correlate with transplant survival, only 5 of the original 20 plots survived through the winter. Low survival with

only expansion at a few sites is regularly reported during seagrass restoration (Suykerbuyk et al. 2016; Paulo et al. 2019) and can be associated with stochastic events such as winter storms (van Katwijk et al. 2016). All our surviving plots, however, originated from a single site. At this site, percentage glued transplants was considerably lower than measured at the other sites ($39.7 \pm 8.3\%$ compared to $82.7 \pm 5.8\%$). *P. dumerilii* is known to reproduce and peak in numbers during the winter (Bedford & Moore 1984) which may have resulted in increased gluing and decreased survival at locations with high ragworm numbers.

Restoration Strategies

Although seed-based restoration has proven to be successful in some systems (Orth et al. 2020) and may scale easier due to lower costs and lower impact on the donor populations, we found it to be unsuccessful in Lake Grevelingen. Despite optimization of donor locations and timing of seeding, early life stage bottlenecks were too limiting for seed-based restoration to succeed. In environments like Lake Grevelingen with strong bottlenecks for the early life stages of seagrass—in our case particularly hydrodynamics and bio-turbation by lugworms—we suggest it may be more effective to avoid these bottlenecks altogether by using transplants rather than seeds. The potential discovery of a new bottleneck for adult plants poses an issue for transplant-based and seed-based restoration alike in our study system. More generally, the interaction between *P. dumerilii* and *Z. marina* contributes to the increasing body of literature stressing the importance of interspecific interactions for seagrass restoration and conservation, especially in degraded systems (Davis et al. 1998; Valdemarsen et al. 2011; Infantes et al. 2016). With coastal food webs changing through anthropogenic stressors (Moksnes et al. 2008; Baden et al. 2012; Riera et al. 2020), previously weak interactions can become more prominent (Maxwell et al. 2017). While habitat suitability assessments are now often the first step in seagrass restoration (van Katwijk et al. 2000; van der Heide et al. 2009; Folmer et al. 2016), these typically focus on abiotic interactions. We suggest that inclusion of both trophic and non-trophic interactions is a vital next step to expand the applicability of such models.

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Supporting Information

The following information may be found in the online version of this article:

Figure S1. Setup in Lake Grevelingen of the seed- and transplant-based restoration experiments.

Table S1. Overview table containing mean (SE) environmental variables per site.

Table S2. Result table with significant test statistics from the analyses of the GLM models on site effects and environmental variable effects.

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