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RESEARCH ARTICLE

Improving seed-based restoration in Eelgrass (*Zostera marina*) in combined aquarium and field experiments

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Abstract

Introduction: Seagrass restoration efforts are being implemented globally to accelerate the slow natural recolonization success. Among the various existing methods, seed-based approaches offer the greatest scalability for species like *Zostera marina*, yet these methods remain undeveloped for a number of sub-tidal systems like the Baltic Sea.

Objectives: To address this, parallel field and lab experiments were conducted to examine the effect of six factors on *Z. marina* seed germination and establishment.

Methods: Laboratory experiments focused on seed coat color, collection site, temperature, and salinity, while field studies explored seed coat color, sowing timepoint, and *Arenicola marina* bioturbation.

Results: Seed coat color and salinity emerged as the two most significant factors influencing germination success. Gray seeds consistently outperformed dark and light seeds by two and 14 times in the lab, and two and five times in the field. Sowing time was also important, with seeds sown later in spring germinating faster and achieving higher germination rates (5 ± 2 to 11 ± 3 seedlings from 100 gray seeds sown). In the lab, salinity significantly below natural conditions (5 vs. 18–20 psu) was detrimental to germination success. Despite complex temperature effects (e.g. at 10°C, gray seeds showed both their highest and lowest germination success across different salinities), seeds at 10°C reached their peak germination faster, within 40 days, whereas those at 5°C needed 50 days. Optimal germination occurred under 18–20 psu and 10°C conditions, with a maximum of 45 ± 6 seedlings (out of 100 gray seeds sown). Field germination in combination with early survival was four times lower than in controlled lab conditions.

Conclusions: Our findings suggest that *Z. marina* seed germination can be optimized by sowing them in late spring, aligning with higher temperatures and natural salinity levels.

Implications for Practice: A key challenge in marine restoration is the limited success of natural recolonization in subtidal environments, and the underdevelopment of scalable, seed-based methods for *Zostera marina* in such settings. Key determinants of germination performance determined herein, such as seed selection and timing of sowing, provide practical recommendations for improving and scaling up seed-based restoration efforts in the German Baltic Sea and comparable systems.

Key words: ***Arenicola marina*, Baltic Sea, bioturbation, phenology, salinity, seagrass, seedling, temperature**

Introduction

Seagrass meadow areas have decreased by 19% globally (Dunic et al. 2021), and by one third across Europe in the last 150 years, between 1869 and 2016 (De los Santos et al. 2019), while regional losses, for example, in the Western Baltic Sea are even higher (up to 60%; Baden et al. 2003). Seagrass restoration has grown globally over the past 30 years, with the United States and Australia leading efforts, and European countries only starting a decade ago (van Katwijk et al. 2016; Orth et al. 2020). Two primary methods of restoration have been developed: the single and multiple shoot transplant, and seed-based method. The former has shown great success, yet it can lead to lower genetic diversity (Williams 2001). Clonal spread, the primary mode of expansion in single-shoot transplantation, will replicate identical genet genotypes. The resulting lower genotypic diversity per area can reduce adaptability to environmental changes and increase vulnerability to diseases, potentially affecting the long-term population growth (Williams 2001). On the other hand, seed-based restoration methods have had very mixed success, from spectacular achievements in the case of Virginia lagoons (Orth et al. 2020) to complete failures in northern Europe in earlier years (Eriander et al. 2016; Cronau et al. 2023), and recent successes in intertidal *Zostera marina* populations in the Dutch Wadden Sea (Gräfnings et al. 2023).

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Seed-based restoration offers two crucial advantages for global seagrass restoration over vegetative shoot-based techniques. The approach is easily scalable and possibly also suited for mechanization and automatization (e.g. Orth et al. 2020; Gräfnings et al. 2023). Second, the restored meadow will feature a much-enhanced genetic and genotypic diversity. A higher genetic variation, in turn, may enhance both present-day resilience and adaptability of restored populations to climate change (Reynolds et al. 2013). As a current major obstacle, the seed biology of seagrass species and local populations varies greatly, requiring regionalized seed-based restoration methods (Larkum et al. 2006) which are currently lacking for our target species and area, Eelgrass (*Z. marina*) in the Western Baltic Sea.

The German coast of the Baltic Sea has a marine angiosperm *Z. marina* (*Zostera*, *Alismatales*) coverage of around 285 km^2, making it one of the most important ecosystems in its brackish waters. In many other coastal areas, substantial parts of the original *Z. marina* meadows have been lost over the past years, mostly due to eutrophication (Bobsien et al. 2021), needing large-scale restoration efforts to repopulate the area (Orth et al. 2006b; Boström et al. 2014). Some efforts have been made to develop seed-based restoration in northern Europe, e.g. Sweden (Eriander et al. 2016), yet these have not shown great success, and the method has not been trialed along the German Baltic Sea coast.

Seed-based restoration methods mimic the natural seedling development, which, for seagrass *Z. marina*, starts with a seed dormancy state in the sediment for several months until they emerge from the seafloor as a seedling (Moore & Short 2006). In general, seed germination is triggered by genetic and physiological (hormones) factors that are triggered by external factors, such as changes in salinity and temperature, good anaerobic conditions, and sediment composition (Moore et al. 1993, Orth et al. 2000, Wang et al. 2016). The transition from a dormant to germinating seed has also been found to be affected by seed morphology, e.g. seed coat color (Harrison 1991), seed size, and weight (Smith et al. 2022).

However, optimal ranges for these factors can vary broadly between and, even, within species making seed-based restoration techniques that are applicable in one country, not viable in others (Orth et al. 2000). For example, in the Chesapeake Bay (U.S.A.), seeds germinate immediately in autumn, avoiding high seedling loss from wave exposure, due to insufficient seed burial depth (Moore et al. 1993; Marion & Orth 2012; Marion et al. 2021), yet in Sweden seeds germinate mostly in spring to escape storms and low light and temperatures prevailing in winter (Olesen 1999; Infantes et al. 2016), a pattern influenced by latitude. Unlike in the United States, where rapid autumn germination enables large-scale seed-based restoration, spring-germinating *Z. marina* at the northern edge of its range has so far shown no restoration success outside tidal areas (Eriander et al. 2016; Infantes et al. 2016). This highlights the importance of considering site and latitudinal variations within species when developing restoration strategies, and how crucial it is to adapt restoration regionally.

Seeds from the same seagrass species, *Z. marina*, but collected from different meadows, have shown varying germination success (Orth et al. 2000; Eriander et al. 2016; Smith et al. 2022), suggesting that the site of origin may influence seed quality, ultimately impacting germination and seedling success. Biotic factors, such as predation and bioturbation, also play an important role in seed and seedling survival, which can vary greatly in space and time (Fishman & Orth 1996; Valdemarsen et al. 2011; Blackburn & Orth 2013; Infantes et al. 2016). As well, variations in seed coat colors among seagrass species are associated with different germination success rates (Harrison 1991). As of now, a comprehensive, multifactorial assessment of key factors for a potential seed-based restoration approach in the Western Baltic Sea is lacking.

This study combines aquaria and field experiments to identify factors influencing *Z. marina* seed germination in the Western Baltic Sea, as well as obtain first estimates of early field mortality rates. We tested the effects of (1) temperature, (2) salinity, (3) sowing time, (4) lugworm mound density, (5) seed site origin (seeds originating from different sites, Kiekut and Falckenstein), and (6) seed coat color on germination. Sowing time later into the season was hypothesized to show higher germinated numbers due to increasing water temperatures, speeding up germination and shortening seed loss phases. Salinity and temperature were expected to influence seed germination, with lower salinities (5 psu compared to ambient levels of 18–20 psu) and higher temperatures (10°C compared to 5°C) anticipated to result in higher germination success, as reported in previous studies (Abe et al. 2008; Infantes et al. 2016).

Methods

Flowering Shoot Collections, Seed Development, and Seed Storage

The optimal time for harvesting *Zostera marina* seeds in the Baltic Sea is unknown. To maximize viable seed collection for experiments, 10–20 flowering shoots were sampled weekly and inspected for fertilization. Once most ovules were fertilized, 365 flowering shoots (each holding approximately 100 potential seeds) were collected via snorkeling at 1 and 2.5 m depth from two sites in Schleswig-Holstein, Germany: Kiekut (sheltered, Eckernförde Bay) and Falckenstein (exposed, Kiel Fjord), between 20 July and 3 August 2022.

Shoots were rinsed in seawater at the beach to remove biofouling organisms, then transported in coolers, and rinsed again in GEOMAR. There, they were placed in 400 and 1500 L outdoor containers filled with 20 µm sand-filtered seawater from Kiel Fjord and continuously aerated (Fig. 1B). Half the water was replaced every 3 weeks to maintain quality. By September–October 2022, most seeds had detached from flowering shoots. Sediment from the containers was sieved (5 mm, then 1 mm) to remove debris. Seeds were separated from finer organic matter using seawater stratification, then hand-sorted by site (Kiekut: 27,060; Falckenstein: 12,481) and color (dark: 4065; gray: 29,295; light: 6181).

Color classes were defined by visual inspection (Fig. 1C) and should preferably be quantified by using objective color metrics for improved results.

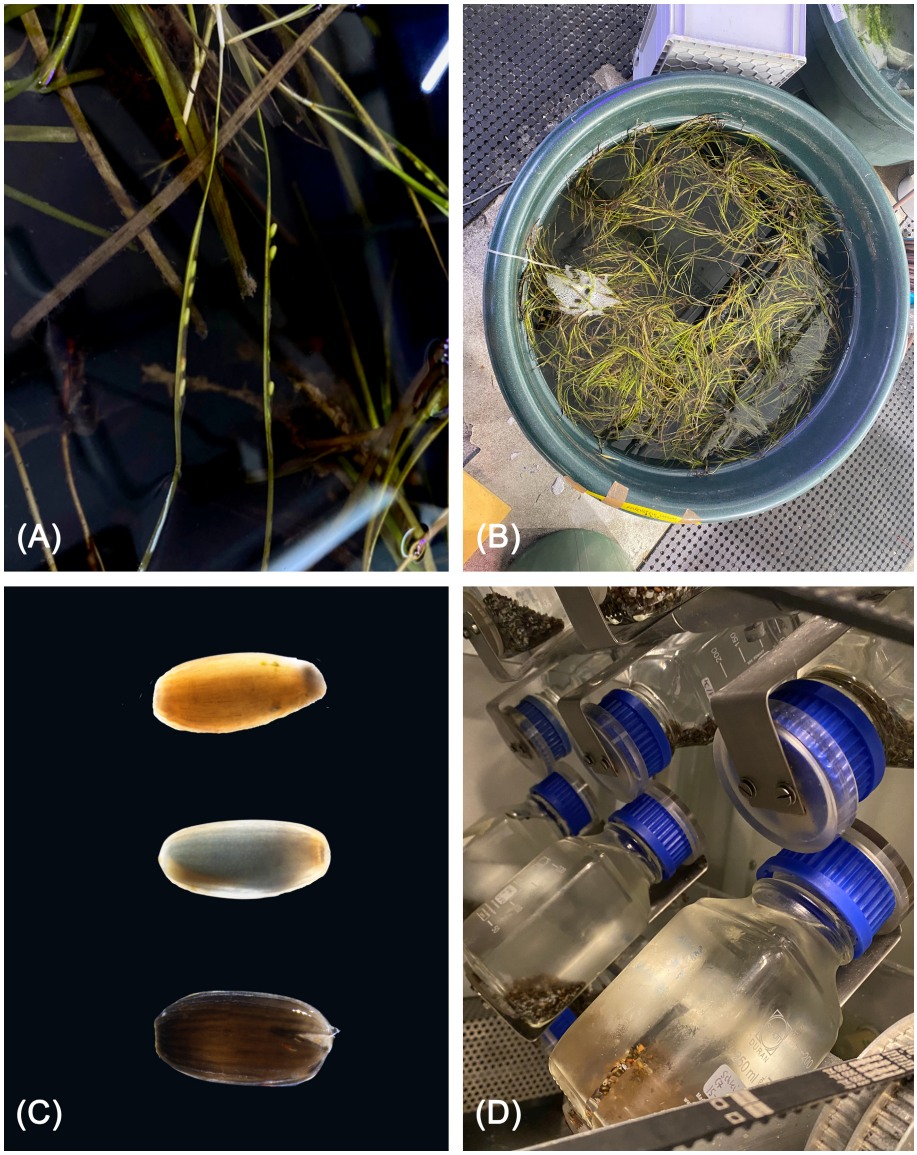


Figure 1. Seed storage and preparation steps prior to sowing them in the field and lab. (A) Seeds in the flowering shoot of *Zostera marina*. (B) Flowering plants were stored in large (400 L) open containers. (C) Seeds were sorted by color: light (top), gray (middle), dark (bottom), and (D) stored in glass flasks inside a climate cabinet (Sanyo versatile environmental test chamber).

Seeds were stored for 4 months in 500 mL glass flasks (2000 seeds/flask) with 30 psu natural seawater at 4°C in total darkness to prevent early germination. Seed quantities per treatment were estimated by weight, based on the average weight of 100 seeds (counted and weighed three times per color).

To standardize conditions, flasks were rotated 180° every 3 hours, and water was replaced weekly (biweekly from December) with pre-cooled, filtered Baltic seawater. Occasional anoxic or sulfidic conditions, caused by decaying organic matter, were not deemed problematic, as prior studies (e.g. Moore et al. 1993; Orth et al. 2006a, 2006b) report enhanced germination under anoxia. Seeds remained in climate-controlled storage until sorting for field and lab sowing (see next sections).

Aquaria Experiment

Experimental Treatments and Parameters Measured. Twelve 60 L aquaria in a climate-controlled room were each divided into six sediment-filled boxes (Fig. S1). Half of the aquaria were set to 5 psu, the rest to ambient (Kiel Fjord) salinity (18–20 psu), at either 5 or 10°C, based on evidence that reduced salinity enhances germination, and to simulate the known effect of increasing germination with higher temperatures (Abe et al. 2008; Infantes et al. 2016). Each box held 100 seeds of one color (gray, dark, and light) from one site (Kiekut and Falckenstein), covering all combinations of treatments (Fig. S2). Salinity, temperature, and seedling counts were recorded five times weekly from 8 March to 1 June 2023, using a salinometer and thermometer.

Aquaria Set Up. Each of the six boxes was filled with natural, autoclaved sediment from Kiel seagrass meadows to eradicate pathogenic bacteria, spores, larvae, and small invertebrates. Aquaria contained two light-emitting diode lights (i.e. Sunset 3500 k and 11,000 k, average light intensity of 68.4 µmol at sediment height) providing 12 hours of light, extended to 13.5 hours after mid-April 2023, and a water stream to prevent stagnation. From 26 to 28 February 2023, boxes were sown with 100 seeds of one color and collection site using the same seed preparation and trench-based sowing method as in the field experiment. All aquaria were maintained at 5°C for acclimation until 6 March 2023, then six were gradually increased to 10°C. Cyanobacteria were controlled with ultraviolet filters as needed.

Field Experiment

For the field experiment layout in the Kiel fjord (Seebar, 54°20′53″N, 10°09′02″E) three 3 × 3 m quadrats (1–1.5 m depth) were each subdivided into 36 plots (50 × 50 cm). A random block design was used to distribute three sowing time points and seed colors (Fig. S3). Seeds were sown by divers on three different time points: 22 February, 16 March, and 13 April 2023. The design mimicked the aquaria setup as closely as possible; however, temperature and salinity were not controlled in the field.

Before sowing, approximately 100 ungerminated seeds (overwintered for 4 months) were placed in flasks half-filled with 16 psu seawater at 4°C, then topped with fresh Kiel Fjord seawater on sowing day for acclimatization. Due to limited seed availability, seeds from both sites were pooled together. Gray seeds were sown across all time points (10 replicates each); light (10 replicates each) and dark seeds (six replicates each) were only sown in February and March.

Each 50 × 50 cm plot received one flask of seeds, sown into a 2–3 cm deep, 30 cm trench at the plot center. After settling, seeds were gently covered and any exposed ones tapped into the sediment (Fig. S4).

From 26 April, until 1 August 2023, *Z. marina* seedlings and *Arenicola marina* (*Arenicola*, *Capitellida*) mounds were counted biweekly by snorkelers, using a 50 × 50 cm frame to delineate the plots (Fig. S4). Temperature and salinity measurements of the Kiel Fjord were taken every 2 days in front of GEOMAR Westshore building (54°19′48″N, 10°08′58″E), approximately 1.5 km from the experimental site.

Germination was defined as the emergence of the plumule, corresponding to the third stage of seedling development, as described by Churchill (1983), and used to assess the viability of the emerging seedling.

Statistical Analysis

All analyses were performed in R v4.4.0 (R Core Team 2021) on Windows 10, with significance set at p less than 0.05.

For statistical significance testing in both aquaria and field experiments, log-logistic, Weibull, or asymptotic regression models were fitted to each individual sample, using the "drc" (Ritz et al. 2015) package, after outlier removal via boxplot. Seedling counts were the response variable and days since sowing the independent variable. The asymptotic seedling number (upper limit of the fitted curve) was extracted for further analysis.

Aquaria experiment. A four-way analysis of variance (ANOVA) tested the interacting effects of seed color, aquaria salinity and temperature, and seed origin on the asymptotic seedling number using the "car" (Fox & Weisberg 2019) package. Assumptions were assessed using Levene's test (homogeneity), Shapiro–Wilk test, and Q-Q plots (normality). Significant effects were further followed by Tukey's honest significant differences (HSD) post hoc test.

Field experiment. A linear model was fitted with log $(x + 1)$-transformed asymptotic seedling numbers (to meet models' assumption) as the response variable, and seed coat color and sowing time as fixed effects.

Due to unbalanced design and absence of interactions between both testing factors, an ANOVA with adjusted sums of squares (type II) was used followed by Tukey's HSD using the "multcomp" (Hothorn et al. 2008) package. Assumptions were assessed using Levene's test (homogeneity), Shapiro–Wilk test, and Q-Q plots (normality).

Despite mild residual non-normality, ANOVA was used as alternative tests violated assumptions or produced non-representative results.

Approximate seed loss rates in the field were estimated by comparing asymptotic seedling numbers across treatments to those from optimally germinated seeds in aquaria (10°C, 18–20 psu), averaged across both origin sites (Falckenstein, Kiekut), serving as a survival baseline.

Results

Aquaria Experiment

Across all seed coat colors, most seedlings emerged under the ambient salinity (18–20 vs. 5 psu) ($p = <0.0001$; Table 1). The effect of temperature, however, was not as straightforward because asymptotic seedling numbers showed inconsistent and not significantly different across temperatures ($p = 1$; Table 1). At 5 and 10°C, seedling numbers of light and dark seed coat colors were similar under both 18 and 20 psu (10 vs. 5°C) and 5 psu (10 vs. 5°C). Gray seeds, however, displayed both their highest and lowest germination success at 10°C (Fig. 2). However, all seed coat colors maintained at high temperatures (10°C) reached their peak germination numbers faster, within 30–40 days, compared to those maintained at low temperatures (5°C), which took 40–50 days (Fig. 2).

Gray seeds were significantly more successful than the dark and light seed coat colors (by two and 14 times), with maximum 39 ± 5 seedlings when grown under ambient salinity (18–20 psu) and high temperature (10°C) conditions (Table 2, Fig. 2). Under the worst treatment 5 psu, 10°C, gray seeds still outperformed dark and light seeds by 2.8 and 10.6 times in

Table 1. Results of a four-way analysis of variance to test the effect of four interacting factors: seed origin (Falckenstein, Kiekut), seed coat color (dark, gray, and light), aquaria salinity (18–20 psu; 5 psu), and temperature (10°C; 5°C) on the asymptotic seedling number in the aquaria experiment. Significance was detected at α less than 0.05. All significant results are in bold font.

	df	*Sum Sq*	F *value*	*Pr*(>F)
Seed origin	1	30	0.30	0.6
Seed color	2	4830	23.8	**<0.0001**
Salinity	1	2270	22.4	**<0.0001**
Temperature	1	0	0	1
Seed origin:seed color	2	71	0.4	0.7
Seed origin:salinity	1	176	1.7	0.2
Seed color:salinity	2	439	2.2	0.1
Seed origin:temperature	1	102	1.002	0.3
Seed color:temperature	2	1	0.004	1
Salinity:temperature	1	57	0.6	0.5
Seed origin:seed color:salinity	2	104	0.5	0.6
Seed origin:seed color:temperature	2	137	0.7	0.5
Seed origin:salinity: temperature	1	1	0.01	0.9
Seed color:salinity: temperature	2	238	1.2	0.3
Seed origin:seed color:salinity:temperature	2	36	0.2	0.8
Residuals	48	4866		

seedling numbers. Dark and light seeds reached a maximum seedling count of 18 ± 3 and 5 ± 2, respectively (Fig. 2). Of all the treatments, light seeds sown in low salinity (5 psu) and low temperature (5°C) conditions had the lowest average seedling count (0.8 ± 0.5 seedlings emerged; blue triangle in Fig. 2).

No significant differences were observed between seed origins ($p = 0.6$; Table 1). A maximum of 45 ± 6 seedlings germinated from Falckenstein seeds maintained at 18–20 psu and 10°C, compared to 35 ± 7 seedlings from Kiekut under the same conditions, marking the highest germination success and therefore benchmark for both experiments.

Interactions between seed origin, seed color, salinity, and temperature were not significant (Table 1).

Field Experiment

By using the same seed material in parallel field and lab experiments, we estimated a 68–97% seed loss magnitude under field conditions compared to maximal germination in aquaria (Fig. 5).

Gray seeds sown in April and dark seeds sown in February showed the highest and lowest germination success, respectively. Seed coat color had a significant effect on the asymptotic seedling number in the field ($p = 0.003$, Table 3). Similarly to the trends observed in the aquaria, significant differences in seedling numbers were observed between gray and light-colored seeds ($p = 0.002$) across all sowing time points. However, seedling production from dark colored seeds did not differ statistically from those of light and gray-colored seeds (Table S1; Fig. 3). Sowing time point was found to be borderline significant ($p = 0.04$; Table 3), yet no statistically significant difference was detected by comparing means of different time points through a Tukey's HSD test ($p = 0.09$; $p = 0.8$; Table S1), which could be explained by the fact that both tests are based on different distributions.

At all time points, seed coat color gray had the highest germination success, ranging between 5 ± 2 to 11 ± 3 seedlings (out of 100 sown), whereas dark and light seeds resulted in a maximum of 6 ± 2 and 2 ± 1 seedlings, respectively (Fig. 3). Seeds sown at a later point in time (e.g. April or March vs. February) resulted in higher seedling counts for all seed coat colors (Fig. 3).

A range of 5–39 *Arenicola marina* lugworm mounds were observed within the planting plots during the entire duration of the field experiment in Kiel Fjord. Overall, a weak negative correlation was observed between seedling germination success and presence of *A. marina* mounds; however, this does not indicate an effect on germination (Fig. 4).

Despite the same number of seeds ($n = 100$) being deployed in the field and lab, seedling counts were almost four times higher in the lab than in the field, estimating a 68% seed loss (Fig. 5). Considering only the highest value in both cases, 27.6 ± 0.9 seedlings were obtained in the lab (gray seeds averaged across both origin sites in aquaria with 18–20 psu and 10°C) versus 6.5 ± 0.8 in the field (Fig. 2 vs. Fig. 3). The highest seed loss estimate (97%, from 12.5 ± 0.3 to 0.4 ± 0.1 seedling) was seen in dark seeds sown in February (Fig. 5). Higher seed survival rates were observed in seeds sown later in time, with dark seeds showing the biggest improvement (3% vs. 40%) and light seeds showing similar survival when sown in February versus March (28% vs. 21%; Figs. 5 & 6).

Discussion

In summary, the most significant influence on germination success and subsequent survival was seed coat color and salinity. Temperature and sowing time also affected germination outcomes, though to a lesser extent, and seed origin and lugworm bioturbation showed no effects.

Seed coat color emerged as a highly influential factor in seed germination success across both field and lab experiments. Gray seeds consistently demonstrated significantly higher germination and subsequent survival rates compared to dark and light seeds. Harrison (1991), found a similar result, observing that 75% green seeds (interpreted as gray colored in this study) germinated, compared to just 38% of brown seeds.

Noteworthy, light-colored seeds were difficult to bury during field deployments, with many buoyantly resurfacing from the sediment. Upon further examination, light-colored seeds and dark-colored seeds weighed slightly less (weight of 100 light seeds: 0.84 ± 0.02 g; 100 dark seeds: 0.85 ± 0.03 g) than their gray counterparts (weight of 100 seeds: 0.90 ± 0.03 g). This suggests that seed pigmentation may influence the overall mass, naturally presenting challenges in these seeds settling to the sediment where they can later germinate. However, these colored seeds also did not perform well in the lab, despite being planted prior to the addition of water to the system. This suggests that

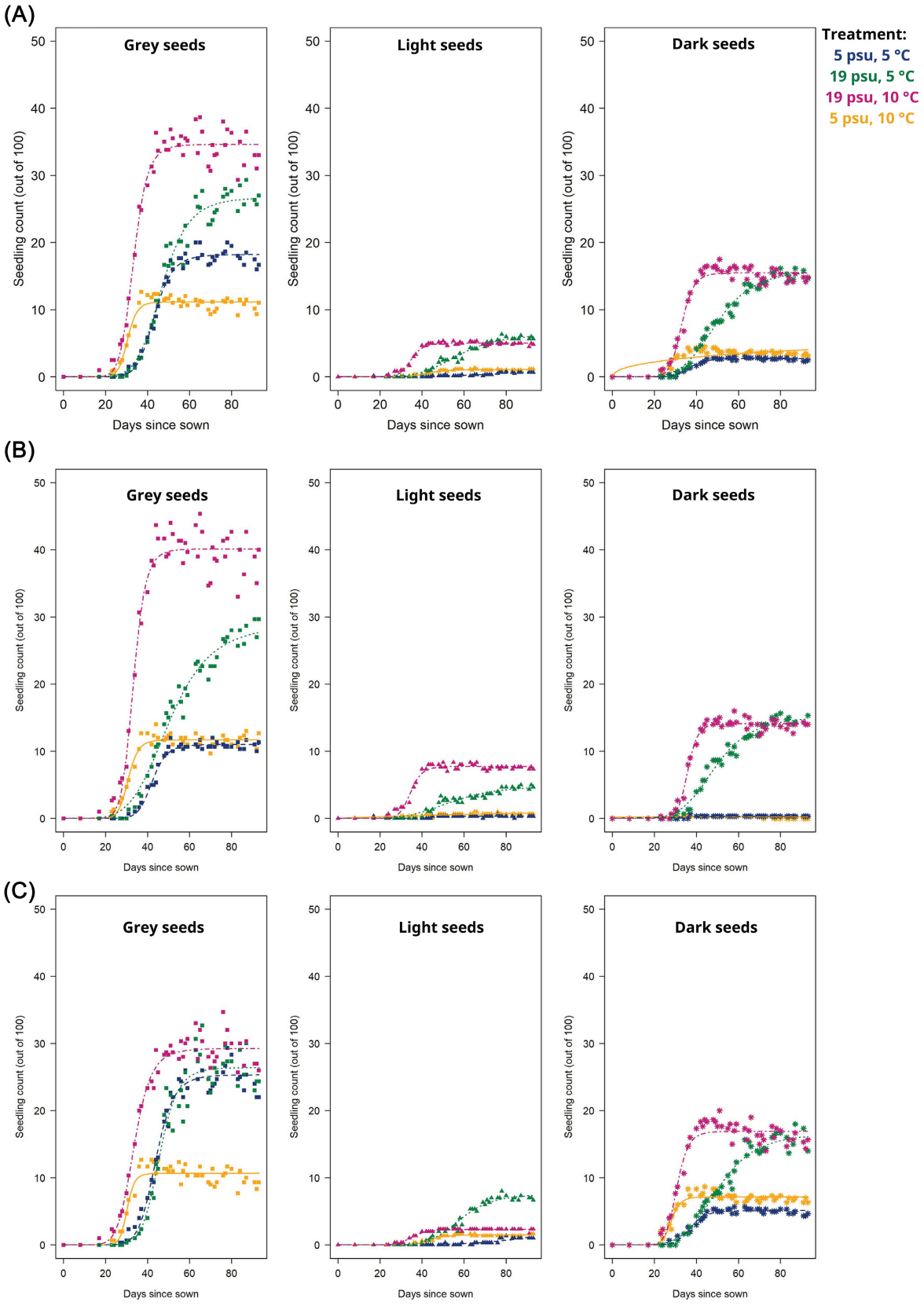


Figure 2. Effect of temperature and salinity on mean seedling count of seagrass *Zostera marina* averaged across two sites (Falckenstein and Kiekut) in Kiel, Germany (A). Site-specific trends can be seen for Falckenstein (B) and Kiekut (C).

seed coat coloration is an indication of impact on both germination and seedling development. Collectively all observations above imply that seed coat color of *Zostera marina* reflects maturity (Harrison 1991), with gray seeds being more likely to germinate compared to the less mature light seeds and the more mature brown seeds. Light-colored seeds may also serve as a bet-hedging strategy by getting widely dispersed as bedload, resembling terrestrial *Pinus* species seeds, that change their morphology (winged vs. non-winged seeds) to adapt their dispersal strategy for different environmental conditions (Salazar-Tortosa et al. 2019). Understanding these differences in seed coat coloration and characteristics can provide valuable insights into their ecology to improve germination outcomes.

Salinity and temperature also helped predict seed germination success in our study. A greater number of seedlings emerged when the seeds were grown under ambient salinity (18–20 psu) conditions and when combined with the high temperature (10°C) treatment. Ultimately, seedling emergence was delayed by lower temperatures (5 vs. 10°C). In other aquarium experiments, seed germination was found to decrease under low temperature conditions and increase under high temperatures (5 vs. 18–20°C; Abe et al. 2008; Infantes et al. 2016).

We expected greater germination success for seeds sown in February than those sown later in time, in March and April, because previous studies indicate that seeds stored for longer periods tend to lose viability (Dooley et al. 2013; Eriander et al. 2016; Sullivan et al. 2022). However, our findings revealed lower, yet not significantly different success in seeds sown in February. Those planted in the field took the longest to reach their highest seedling count, requiring 127–160 days. In contrast, seeds sown in March and April had higher germination success and reached their peak germination counts more rapidly, within 62–138 days. Fast germination and seedling growth are crucial in restoration efforts to limit negative impacts of complex biotic and abiotic mortality sources (see below), suggesting that the latest time point, namely April, with having the highest attained seedling count would be the best sowing period for restoration in the German Baltic Sea.

These temporal differences in germination success align with an increase in temperature and salinity in Kiel Fjord. Here, temperatures increased from 4.8°C in February to 15.4°C by the end of the field experiment, in August (Hiebenthal et al. 2023). The salinity in Kiel Fjord decreased from 20 psu in February 2023 to 16 psu in April 2023, which might explain the higher germination success when sown later in time. Another factor influencing the timing of optimal seed germination and therefore seedling establishment could be burial depths, as shown by Marion and Orth (2012) in the Chesapeake Bay (U.S.A.). Other field studies have shown that optimal germination occurs either in spring (in northern countries) or autumn (Moore et al. 1993; Eriander et al. 2016; Vercaemer et al. 2021). For example, in Scandinavian waters, *Z. marina* seeds germinate in the spring (March and April), after remaining in a dormant state for 7–8 months

Table 2. Tukey's Tukey honest significant differences (HSD) post hoc test to assess significant differences between asymptotic seedling numbers of the seed coat colors (dark vs. gray vs. light). Significance was detected at α less than 0.05. All significant results are in bold font.

Comparison	*Estimated difference in means*	*Lower 95% CI bound*	*Upper 95% CI bound*	*Adjusted* p *value*
Gray versus *dark seeds*	13.3	6.2	20.3	**0.0001**
Light versus *dark seeds*	−6.4	−13.4	0.6	0.08
Light versus *gray seeds*	−19.7	−26.7	−12.6	**<0.0001**

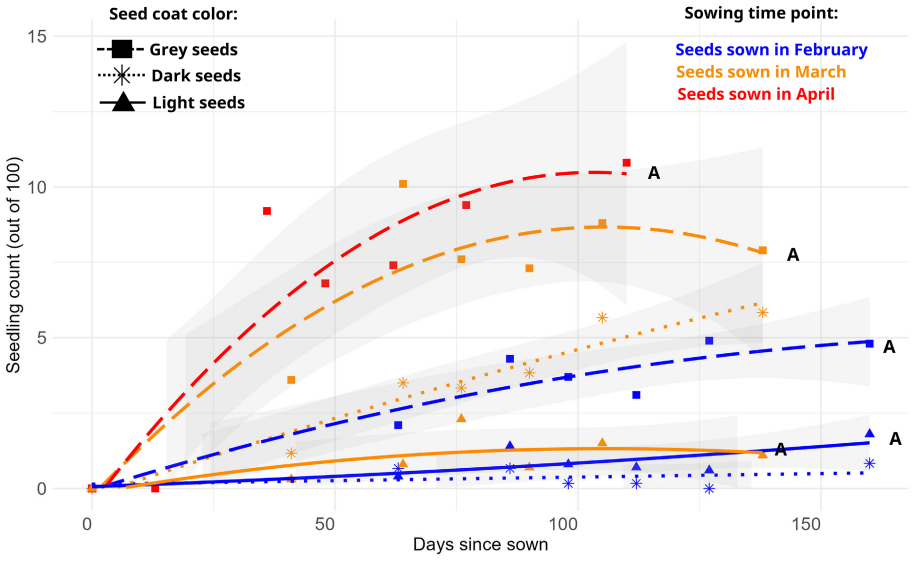


Figure 3. Effect of seed coat color (dark, gray, and light) and sowing time point (February, March, and April) on mean seedling count of seagrass *Zostera marina*, in Kiel, Germany. One hundred seeds sown per treatment. Letter "A" depicting statistical significance. CI = 90%.

(Olesen 1999; Infantes et al. 2016). The long, dark, and relatively cold winters along the German Baltic Sea coast pose significant challenges for seedling growth and survival.

Previous studies have attributed the loss of seeds and seedlings in the field to bioturbation, predation and physical sediment movements; factors they found to limit seed-based restoration efforts (Marion & Orth 2012; Infantes et al. 2016; Jørgensen et al. 2019; Cronau et al. 2023). In contrast to our initial hypothesis, based on these previous findings, non-significant effects of *A. marina* mounds were found on the germination success of seeds sown in the field, likely because the detrimental range of 29 ind./m^2 (Banke et al. 2025) was not yet reached. However, it is important to note that seedling counts were almost four times higher in the lab than in the field, showing a clear estimate of seed loss in the field at approximately 68–97%, a phenomenon observed also in the Chesapeake Bay, showing 80–95% seed germination success in the lab and 10.3–40.4% in the field (Marion & Orth 2012). This suggests that seed loss and therefore also germination success might be strongly influenced by biotic interactions such as bioturbation and predation (Delefosse & Kristensen 2012; Blackburn & Orth 2013; Infantes et al. 2016). In addition, abiotic factors such as sediment movement through hydrodynamics created by storms make for significant loss of seeds due to insufficient seed burial depth (Marion & Orth 2012; Infantes et al. 2016; Marion et al. 2021).

Conversely, some studies demonstrated a positive effect of seed burial by *A. marina*, as in some instances they provide the seed with optimal germination conditions (Blackburn & Orth 2013). A deeper exploration into biotic factors may be essential to fully understand the challenges associated with seed deployments in the field and assess the effectiveness of seed establishment under a diverse range of biological interactions.

In our study, no significant differences in germination success were observed between seeds collected in Falckenstein compared to Kiekut, two sites that are about 35 km distant from one another along the coastline. However, other studies have observed site-specific differences in seagrass seed germination success, highlighting changes in life-history strategies that may result from adaptations to local conditions (Orth et al. 2000; Smith et al. 2022). Eriander et al. (2016) found that *Z. marina* seeds harvested from exposed bays had better seedling establishment and growth compared to those collected from sheltered bays. In this context, wave exposure was measured for both sites, following Schubert et al. (2015), showing that Kiekut (38.4 W) is about half as exposed as Falckenstein (81.1 W), yet well below the average for the Schleswig-Holstein coast (604 W). This may contribute to its slightly higher, yet not statistically significant germination success (by 1.3 times), suggesting that environmental exposure could play a role in influencing seed performance, but the precise controls of seed germination throughout this species range remains unclear.

Additional considerations for optimizing seed-based restoration methods include further research into the interactions

Table 3. Analysis of variance with adjusted sums of squares (type II) performed on a fitted linear model to test the effect of seed sowing time and seed color on the log $(x + 1)$-transformed asymptotic seedling numbers in experimental plots in Kiel Fjord, Germany. Significance level, α less than 0.05. All significant results are in bold font.

	df	*Sum Sq*	F *value*	*Pr(>F)*
Seed color	2	13	6.6	**0.003**
Seed sowing time point	2	6.7	3.4	**0.04**
Residuals	57	56		

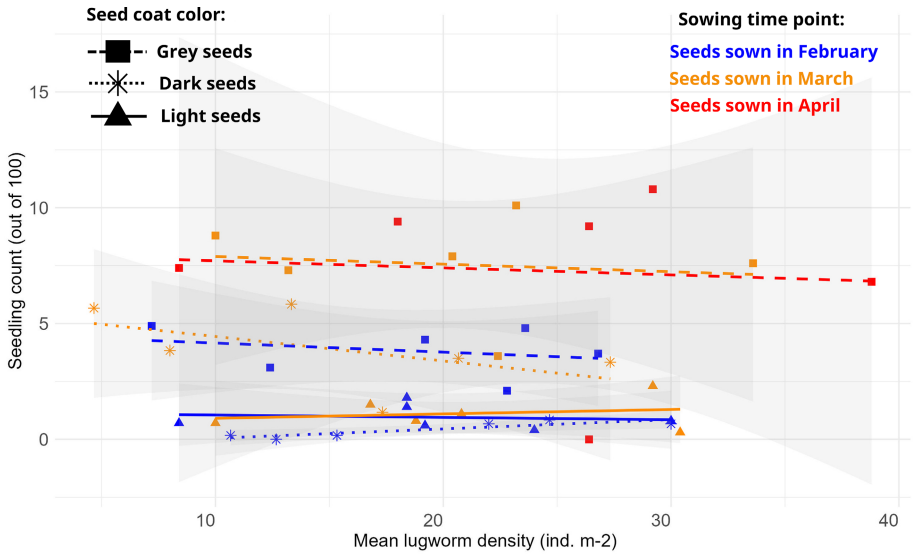


Figure 4. Correlation between lugworm *Arenicola marina* mounds and mean seedling counts. Correlations are outlines for different seed coat colors (dark, gray, and light) and sowing time (February, March, and April). Error margins represent 90% CIs.

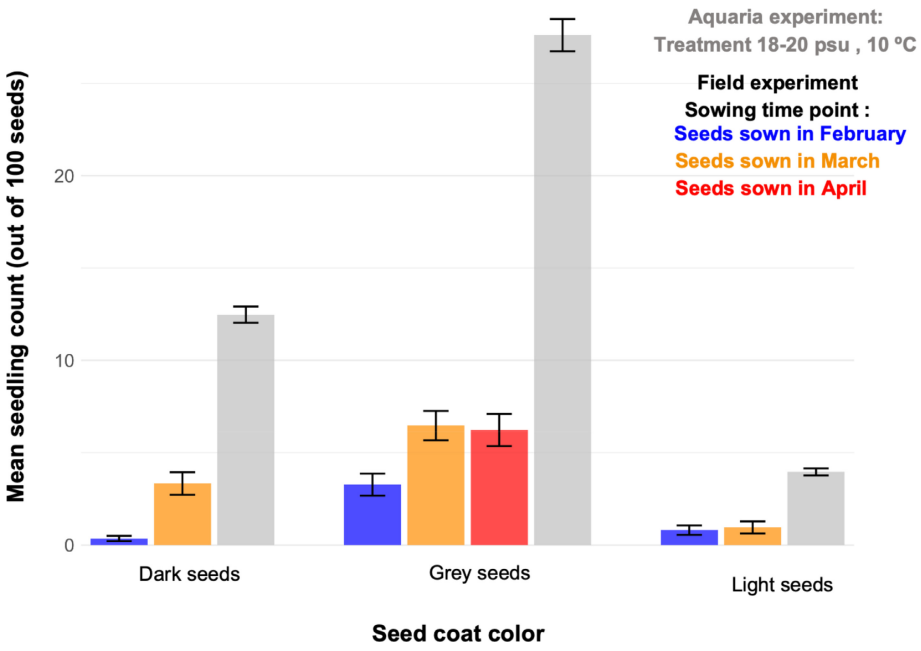


Figure 5. Estimated seed loss rates in the field, based on mean seedling count of different treatments (seed coat colors: light, gray, and dark/seed sowing times: February, March, and April), compared to baseline germination under optimal aquaria conditions (10°C; 18–20 psu). The baseline germination values were averaged across both seed origin sites.

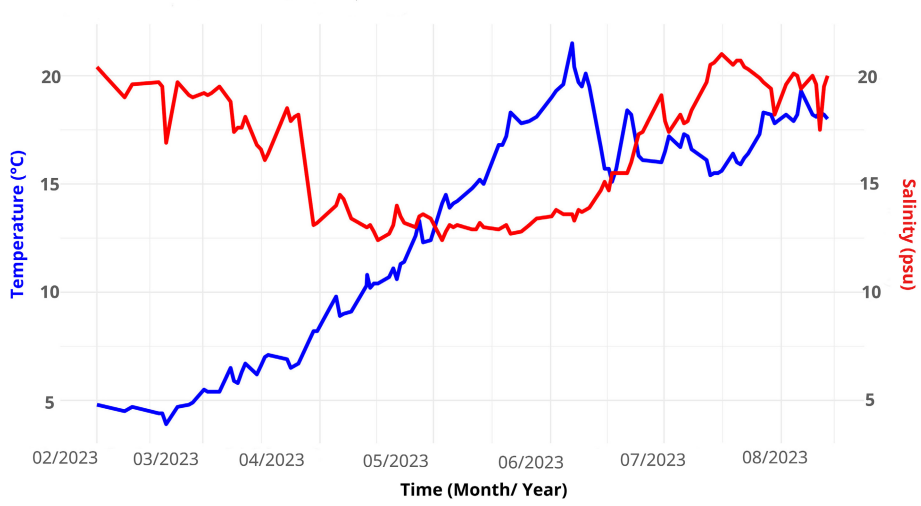


Figure 6. Temperature (°C) and salinity (psu) in Kiel Fjord, where the field experiment took place from February to April 2023.

between seed origin and the suitability of seed material for restoration. Seeds sowing should occur later into the season to speed up germination with increasing temperatures. To maximize germination success, seeds should be sown under ambient water salinity conditions. Understanding seed loss variables could help determine the number of seeds needed to upscale restoration efforts. Sorting seeds by color is currently too time-consuming and challenging, and of no advantage, since gray seeds are already dominant and show higher germination success. However, the use of a flow-through seawater flume, as demonstrated by Marion and Orth (2010) could help remove less mature seeds (e.g. light seeds) and therefore increase germination success. Additionally, seagrass meadow seed-based restoration in the Baltic Sea can maximize local genotypic diversity and therefore enhance both present day resilience and adaptability of restored populations to climate change (Reynolds et al. 2013).

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Supporting Information

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

Figure S1. Aquaria layout in climate-controlled room.
Figure S2.. Schematic of multi-factorial aquaria layout in climate-controlled room.
Figure S3. Schematic of the random block design of the field experiment in Kieler Förde, Schleswig-Holstein, Germany.
Figure S4. Seed sowing process and data collection in the field experiment.
Table S1. Tukey's honest significant differences (HSD) post hoc multiple pairwise-comparison test.

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