

# Using marsh organs to test seed recruitment in tidal freshwater marshes

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**Abstract**

**Premise:** Seed recruitment niches along estuarine elevation gradients are seldom experimentally field-tested under tidal regimes of the Pacific Northwest of North America. Addressing this knowledge gap is important to better understand estuary restoration and plant community response to sea level rise.

**Methods:** Germination was tested in marsh organ mesocosms across an elevation gradient (0.5–1.7 m above mean sea level). Seeds were sown on sterile peat moss, and the tops of pipes were secured with horticultural “frost cloth” to ensure no experimental seeds were washed out and no new seeds were introduced. The trials tested artificial and overwinter chilling regimes, as well as the presence and/or absence of a near-neighbor transplant.

**Results:** *Carex lyngbyei* had significant elevation-driven germination after overwinter and artificial chilling. *Schoenoplectus tabernaemontani* had near-significant germination across elevation after overwinter chilling, and germination in the absence of competition was significantly greater than with a near-neighbor transplant.

**Discussion:** *Carex lyngbyei* had the highest germination rate at higher elevations, which suggests restricted seed recruitment potential and required clonal expansion to extend into lower marsh elevations. Identifying species-specific recruitment niches provides insight for restoration opportunities or invasive species monitoring, as well as for estuary migration under sea level rise.

**KEYWORDS**

climate change, coastal resilience, managed retreat, salmon habitat, tidal freshwater marsh, windows of opportunity

Plant community composition in estuaries is driven by abiotic environmental gradients such as salinity and inundation duration, which are in part functions of elevation above mean sea level (Ewing, 1983; Bertness and Ellison, 1987). Tidal freshwater marshes (TFMs) are upper reaches of the estuary where salinity influence is low (<0.5 parts per thousand [ppt]) due to the prevalence of freshwater from outflowing streams or rivers (Cowardin, 1979; Odum, 1988). These habitats serve as vital near-shore habitat for migratory salmon and seabirds (Magonigal and Neubauser, 2009; Chalifour et al., 2019). They also provide high levels of ecosystem services, such as carbon storage and nutrient cycling, disproportionately greater than their area (Mueller

et al., 2016). Consequently, loss of TFMs largely driven by urbanization results in loss of vital habitat and ecosystem services (Lee et al., 2006). These effects are compounded by sea level rise, as TFMs will be “squeezed” between impervious urban infrastructure and rising tide heights, thus restricting the amount of area available for habitat provisioning or carbon sequestration (Torio and Chmura, 2013; Kauffman et al., 2020). In recognition of their valuable contributions to habitat and ecosystem services, there has been an increased interest in restoring TFMs, especially in North America and Eurasia (Middleton, 1999; Ebberts et al., 2018; Gailis et al., 2021). Restoration initiatives for TFM habitat highlight the need for better understanding of the processes that determine

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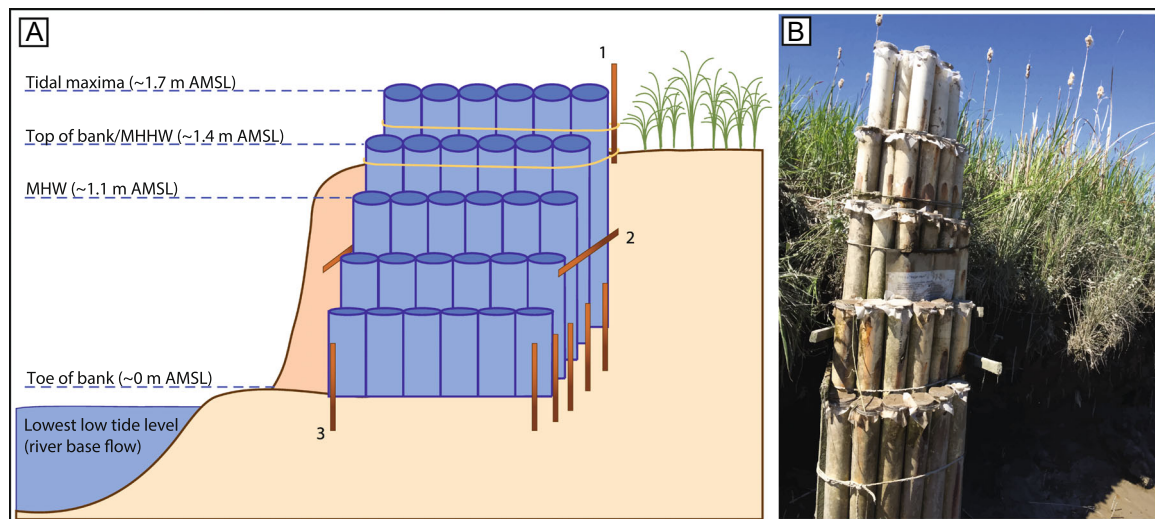
community assembly and succession over time. These initiatives also require the development of strategies that opportunistically use natural drivers of diverse, resistant, or resilient habitats to meet practitioner objectives (e.g., Beechie et al., 2010), such as the natural recruitment of plants.

Many of the plant species in TFMs are perennial and typically reproduce clonally, including those in the Pacific Northwest of North America (Emmett et al., 2000). This enables population and community expansion that can withstand environmental risks and stressors found in estuarine habitats (Hopfensperger et al., 2009; Buffington et al., 2018). A species' occupancy range within tidal marshes is bounded by its intolerance to abiotic factors such as salinity or inundation at lower elevations, and by competitive interactions at upper elevations (Bertness, 1991). These community patterns of stress tolerance have been well characterized in clonal populations (Bertness and Ellison, 1987; Callaway et al., 2012; Borde et al., 2020); however, new genetic diversity must be introduced by sexual reproduction, which is achieved in plant communities through the successful establishment of new plants from seeds.

Existing plants can facilitate disturbance-free space by reducing wave stress or increasing soil oxygen to provide suitable micro-habitats for seedlings (Reddy and DeLaune, 2008; Vogt et al., 2014), although they may also compete for light and nutrients. In tidal marsh studies on the east coast of North America, plants exhibit more facilitation in higher stress environments (Bertness and Hacker, 1994); however, studies focused on the west coast of North America have shown these interactions are absent or variable under high salinity stress (Keammerer and Hacker, 2013; Noto and Shurin, 2017). In general, studies

of the recruitment of plants from seeds in TFMs are less common than studies of mature populations (but see Rand, 2000; Keammerer and Hacker, 2013). Understanding plant life cycle processes, and competitive interactions between life stages, is an unaddressed knowledge gap in these habitats. Inferences about tolerance of mature plants to tidal inundation and salinity stress have, in part, been determined using transplants in “marsh organ” mesocosms (Figure 1) to experimentally test effects of elevation gradients on successful transplant establishment while exposing plants to all other environmental variables (Janousek et al., 2016). This method represents an opportunity to conduct field experiments of seed germination; however, the marsh organ approach has not yet been used in this context.

In TFMs, a critical environmental gradient is the duration of daily tidal inundation to which newly germinated seedlings are subjected (Peterson and Baldwin, 2004). Tides in the Pacific Northwest follow a semi-diurnal tide cycle with daily mean high water (MHW) and mean higher high water (MHHW) levels. Communities of mature TFM species predominantly occupy tidal elevations between the MHW and MHHW elevation profile of the marshes, although ranges can extend beyond these boundaries (Janousek et al., 2019). Elevations above the MHHW level have the longest inundation-free periods for germination for emergent species, as they are only flooded on monthly tidal inundation maxima or “spring” tides. River discharge decreases through the later summer in snowmelt-driven watersheds (Kostaschuk, 2002), lowering the total depth of the water column so that more of the marsh profile escapes tidal inundation, thus extending the inundation-free space for germination. Therefore, elevations approximately at or above



**FIGURE 1** (A) Schematic of marsh organ structure against the riverbank to capture a range of tidal inundation. Elevations at or below the mean high water (MHW) level are inundated daily; mean higher high water (MHHW) elevations are inundated approximately every two weeks; and elevations at the tidal maxima receive inundation for 1–3 days monthly during June and July. (1) A wooden stake at the top of the bank provides an anchor for stabilizing the top rows of the pipes. (2) Stakes positioned into the side of the bank provide resistance to shear stress. (3) Stakes bracing the pipe bases provide stability and eliminate the need to drive the PVC pipe deeper into the ground. Figure not drawn to scale. (B) Marsh organ at the field site at low tide. AMSL, above mean sea level. Photo by S. Lane, 2021.

the MHHW level provide a “window of opportunity” for a relatively stress-free recruitment niche for seeds to germinate and develop compensatory anatomies such as root aerenchyma to withstand the inundation stress of subsequent tidal events (Balke et al., 2011; Silinski et al., 2016). In addition to sufficient windows of opportunity, seeds often require a dormancy period only broken after exposure to freeze/thaw cycles (e.g., Thullen and Eberts, 1995). Therefore, the use of seeds in field studies must account for this by either allowing seeds to overwinter at the field site or providing an artificial chilling period. Although it is possible to simulate dormancy release through artificial stratification, this presents a potential source of confounded interactions in germination experiments that seek to test natural processes (Schütz and Rave, 1999).

The main objective of this study was to determine whether marsh organs can be adapted to field-test seed germination across a range of tidal inundation elevations. As part of this objective, I sought to test whether artificially chilled or naturally chilled conditions affected germination rates in the experimental mesocosm. If seedlings are able to withstand inundation stress soon after germination, they can be expected to germinate over a broad range of tidal elevations. Alternatively, if compensatory anatomies such as aerenchyma or clonal growth are required to mitigate inundation stress and expand the population to lower elevations, the seeds of all species tested would then be expected to germinate only at the highest elevations. A second objective was to test whether germination rates of seeds would be facilitated or competitively excluded by near-neighbor plants. If facilitative interactions promote the recruitment of seeds by reducing abiotic inundation stress, then germination rates in trials with a neighbor plant should be the same or greater than in trials without a neighbor. Inferences from these experiments can be used to identify

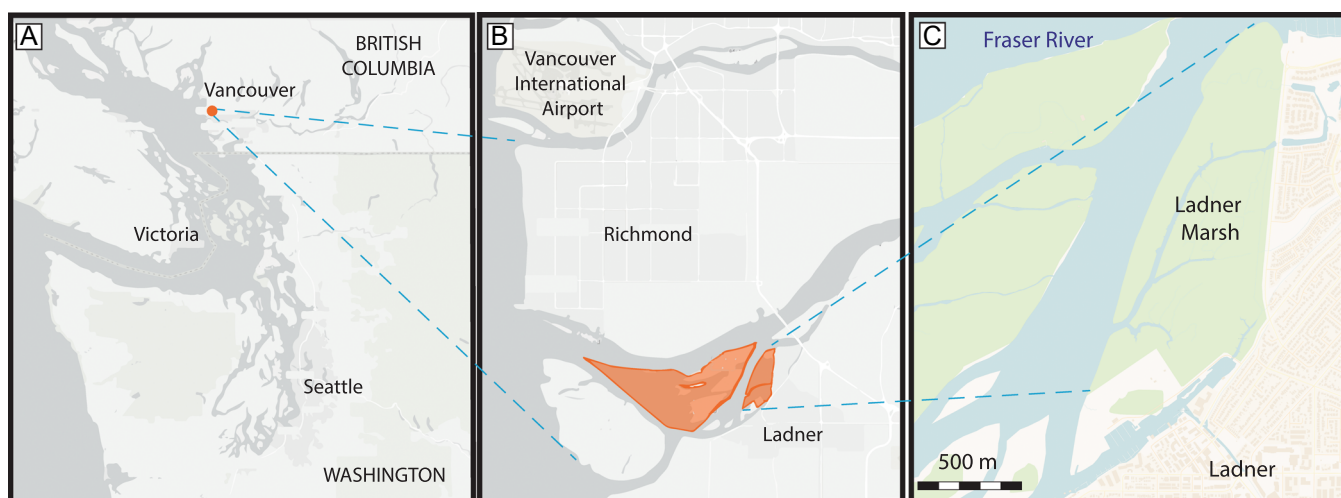
likely recruitment niches under different inundation regimes and competitive conditions, which can help inform practitioners interested in using seed restoration techniques in TFM communities.

## METHODS

### Site and tidal context

Ladner Marsh is part of the South Arm Marshes Wildlife Management Area approximately 12 km upstream of the mouth of the Fraser River, in British Columbia, Canada, where semi-diurnal tide cycles result in local tidal amplitudes of ~4 m (Figure 2). Four site locations within Ladner Marsh were selected for equal exposure to inundation from the Fraser River and along an interior tidal creek. Salinities at the surface of the water column in the marsh vary with the flow rate of the Fraser River and amplitude of the saline tidal wedge. Freshwater inundates the marsh platform during the growing season as May–July snowmelt runoff (“freshet”) results in greater river discharge ( $4000\text{--}8000\text{ m}^3\text{ s}^{-1}$ ), preventing tidal mixing and restricting the saline tidal wedge to the river bottom (Milliman, 1980; Kostaschuk, 2002). As freshet subsides, discharge rates decrease to an annual minimum ( $<2000\text{ m}^3\text{ s}^{-1}$ ) during the winter months (January–February) into early spring (April), which allows the saline tidal wedge to mix more freely with river flows, resulting in brackish ( $<30\text{ ppt}$ ) surface waters that overtop marsh platforms during vegetation dormancy (Milliman, 1980; Bradfield and Porter, 1982).

Local tides above mean lower low water level (MLLW) were obtained from an Environment Canada hydrometric station ~8 km downstream of the site (Station 08MH028), which reports tide measurements in near-real-time. These



**FIGURE 2** Location of the study site in Vancouver, British Columbia, Canada (A), approximately 20 km north of the South Arm Marshes Wildlife Management Area (highlighted in orange in B). Ladner Marsh abuts municipal development on the south bank of the Fraser River (C). Base maps (A,B) generated by iMap (B.C. Conservation Data Centre, Victoria, British Columbia, Canada; <https://maps.gov.bc.ca/ess/hm/imap4m/>) and (C) OpenStreetMap (<https://www.openstreetmap.org/>).

data were related to site-specific elevation above mean sea level (AMSL) by referencing elevations obtained with a surveyor's level (LineSite AL28 Auto Level; Trintec Distribution Inc., Burnaby, British Columbia, Canada) to a nearby Survey Canada Geodetic Control Marker (GCM 737197, Tablet Marking 84H0431, CVD28 Datum). In Ladner Marsh, 0 m AMSL was ~2.3 m above MLLW. Elevations on the marsh platform were 1.2–1.4 m AMSL (top of bank), and elevations at the toe of the bank were –0.06–0.4 m AMSL. The MHHW level during the growing season (1 March–31 August) was approximately 1.4 m AMSL, and the MHW level was approximately 1.1 m AMSL. The entire marsh platform was inundated when tide heights exceeded the MHHW level; inundation at 1.7 m AMSL occurs approximately monthly during the active growing season (Figure 1).

### Marsh organ design and construction

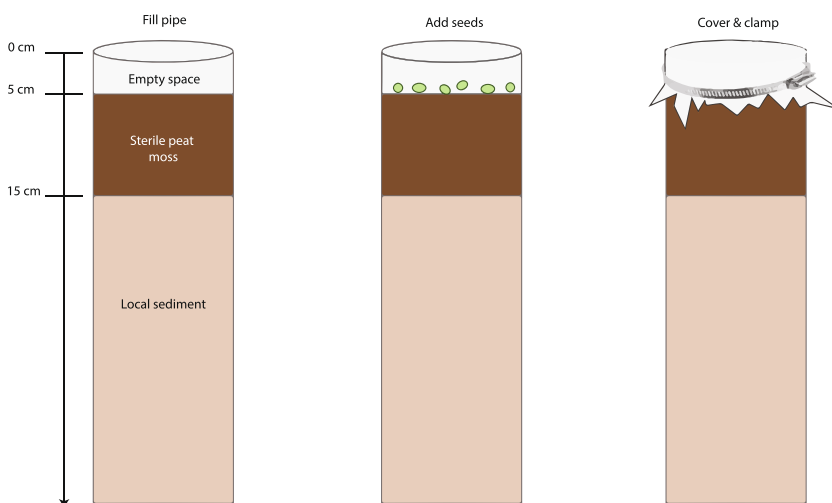
Construction and experimental elevations were limited by the elevation of the toe of the bank (approximately 0 m AMSL). Marsh organs were constructed so that the tops of all pipes were 0.5, 0.8, 1.1, 1.4, and 1.7 m AMSL, which represents the elevation gradient from the current approximate sea level to the monthly tidal maxima.

Marsh organs were constructed by placing 10-cm diameter, Schedule 40 PVC sewer pipe (IPEX HomeRite Products, Oakville, Ontario, Canada) perpendicular to the ground, and driving it 10–15 cm into the marsh substrate. For this experiment, there were six replicate pipes at each of the five tide heights. The highest row of elevation tested was braced tightly against the marsh bank for stability, and pipes on the outer perimeter of the structure were braced with a 2.5 × 5-cm wooden stake. The structure was braced on either side with 2.5 × 5-cm stakes into the marsh platform to resist shear stress, and all marsh organ pipes were anchored to wooden stakes with a cotton rope (Figure 1). Each marsh organ pipe was perforated once every 20 cm along its length with 1.6-mm holes to allow some water to drain.

Each marsh organ pipe was filled within 15 cm of the open top with local sediment substrate from the creek bed. The inner walls of the PVC pipe were then rinsed with clean tap water, and the top 10 cm filled with horticultural peat moss to provide a germination barrier against any existing seeds in the local substrate. This substrate was chosen for its moisture retention, seed-free qualities, and relative ease to obtain and transport into the field. An alternative option was removing, sterilizing, and replacing local substrates; however, this presents additional time and resource challenges, particularly by causing the hardening of clay sediments and loss of fine texture. Immediately after adding experimental seeds, each marsh organ pipe opening was covered with a 15 × 15-cm piece of horticultural spun-bonded polypropylene, medium-weight “frost cloth” (Home Gardener Frost Blanket; Home Hardware Stores Ltd., St. Jacobs, Ontario, Canada). This material was selected because it is permeable to water and air, and allows at least 70% light transmittance when tested with a photometer. The material covering was clamped in place using a 10-cm plumbing ring clamp. This ensured that all seeds added to the marsh organ pipe would be free to float and redistribute in the water within the pipe as they would naturally on the marsh platform, and prevented the introduction of local seed sources into the experimental microcosm (Figure 3). Data loggers (Thermochron iButton; iButton Link LLC, White-water, Wisconsin, USA) were waterproofed and placed on the substrate surface inside the marsh organ pipe to record hourly temperatures within the marsh organs during June and July 2020. Temperatures were recorded at the lowest, middle, and highest tide heights (0.5, 1.1, 1.7 m AMSL, respectively), and at the outermost (upstream, downstream) and the left-center pipes.

### Planting trials

A total of five native TFM species were chosen for their prevalence within the local and regional TFM habitat and their commercial availability. Lyngbye's sedge (*Carex lyngbyei*



**FIGURE 3** Schematic for filling marsh organ pipes with sediment and securing seeds with frost cloth and ring clamp to prevent the unintentional introduction of seeds from the environment or the loss of experimental seeds. Figure not drawn to scale.

Hornem.), bulrush (*Schoenoplectus tabernaemontani* (C. C. Gmel.) Palla), common rush (*Juncus effusus* L.), common cattail (*Typha latifolia* L.), and spikerush (*Eleocharis palustris* (L.) Roem. & Schult.) were obtained from a certified native plant grower (NATS Nursery Ltd., Langley, British Columbia, Canada) specializing in locally sourced ecotypes. Because native populations were not sampled as part of this experiment, specimen vouchers were not included in the data collection.

Seeds were visually inspected to ensure embryo fill and seed viability, and counted into batches of 20. One species, *S. tabernaemontani*, required scarification (Thullen and Eberts, 1995), which was accomplished by brushing seeds on extra fine grit-sized sandpaper (360 CAMI/25.8- $\mu$ m-diameter grit) on a hard, level surface. Prepared seeds were then cold-stratified in water at 4°C for eight weeks to achieve dormancy release by artificial chilling (e.g., Harris and Marshall, 1960; Jones et al., 2004).

Artificially chilled seeds were sown in early June 2020. Germination counts were recorded on 20 July 2020, and germinated individuals were allowed to remain in place. The same trial was re-assessed on 20 August 2020 to measure changes in survival or recruitment timing. Following observations of artificially chilled seeds, the top 20 cm of the marsh organs were cleaned out, replaced with new peat moss, and resown in August 2020 with dry seeds stored at room temperature to test natural chilling of seeds over the winter season; germination was recorded in mid-May 2021. In both these trials, one marsh organ pipe at each tide height was prepared as a negative control.

To test competitive interactions on seed germination, two species (*S. tabernaemontani* and *C. lyngbyei*) were chosen to test germination under three competitive interactions. Seeds of these species were artificially chilled at 4°C for eight weeks and sown in May 2021 under conspecific, heterospecific, or no competition with a single 5-cm diameter, 10-cm root depth, ~10-cm-tall transplant (“plug”) of either a *S. tabernaemontani* or *C. lyngbyei* plant. All pipes were loosely covered with frost cloth positioned so that aerial vegetation was not broken, and clamped securely so that only seeds placed inside the pipe would be observed. Germination was recorded in late June 2021, and any germinated individuals were allowed to continue growing. The same trial was re-assessed in late July 2021 to record changes in survival or recruitment.

## Statistical analyses

All statistical analyses were performed in R version 4.0.2 (R Core Team, 2022). Germination was recorded as a percentage, and data were square root-transformed if necessary to meet test assumptions. One-way analysis of variance (ANOVA) and analysis of covariance (ANCOVA) using height AMSL (m) as a covariate were performed (function *anova\_test*, package ‘rstatix’), and post-hoc Tukey’s test (function *TukeyHSD*, package ‘stats’) was used

to test for significant differences in germination across each tidal height. Two-way ANOVA was used to test temperature differences at various tide heights and pipe positions within the marsh organ.

## RESULTS

Results for species with observable germination are reported. *Eleocharis palustris* (spikerush) and control microcosms did not have germination and are not reported here.

### Germination after natural chilling to break dormancy

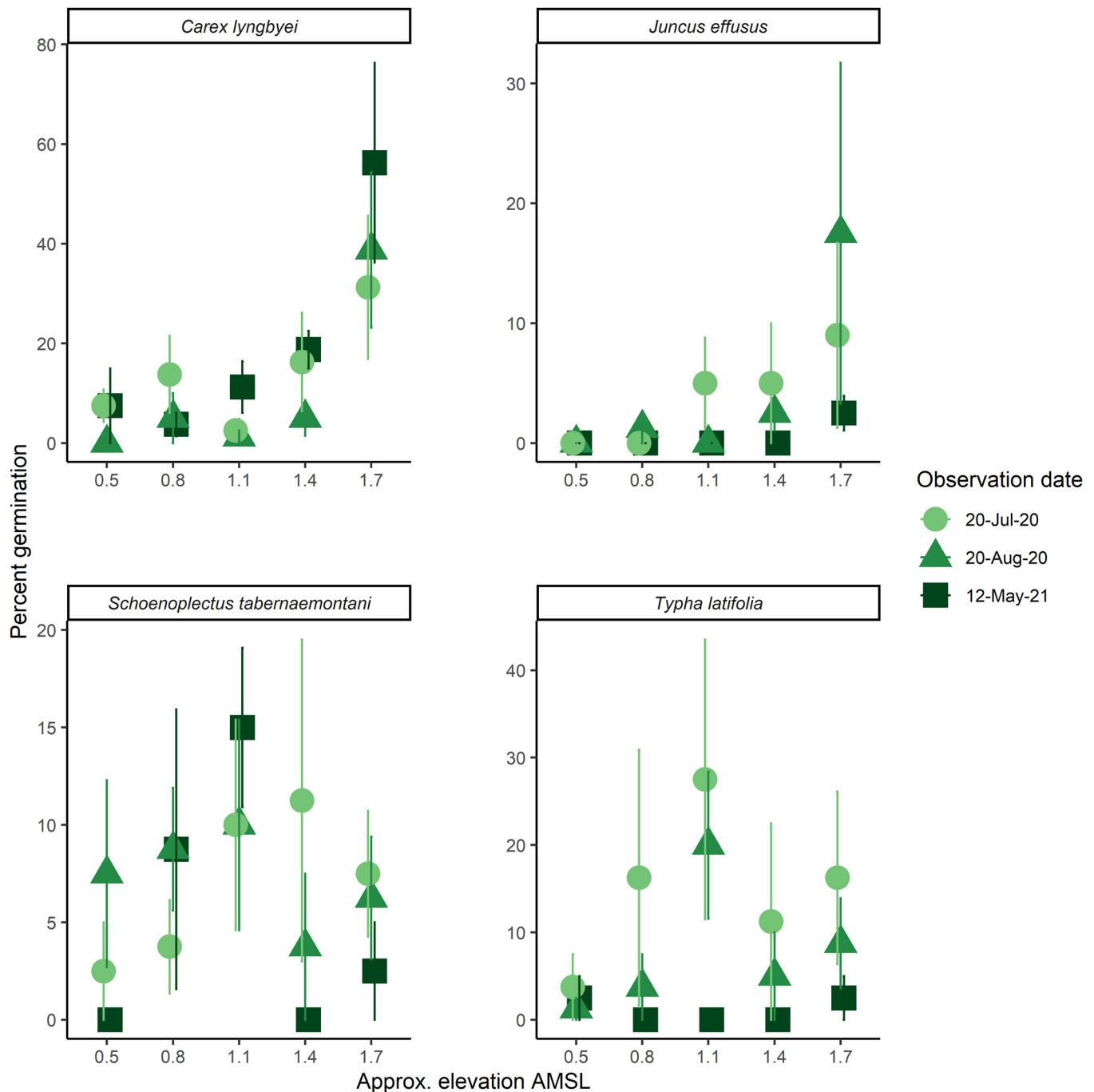
*Carex lyngbyei* and *S. tabernaemontani* had the highest germination rates following natural overwinter chilling, with overall germination trends across all elevations comparable to those observed following artificial chilling the prior summer (Figure 4). *Carex lyngbyei* germination showed a significant difference between the tide heights (ANOVA,  $F_{4,15} = 4.491$ ,  $P = 0.014$ ), with germination at the highest tide height significantly greater than germination in pipes at 0.5 ( $P = 0.026$ ), 0.8 ( $P = 0.016$ ), and 1.1 ( $P = 0.043$ ) m AMSL as tested by Tukey’s HSD.

Germination rates for *S. tabernaemontani* at the tested tide heights were nearly significantly different (ANOVA,  $F_{4,15} = 2.853$ ,  $P = 0.061$ ), and the trend of higher germination rates around 1.1 m AMSL may be strong enough to suggest further tests of germination at tide heights around the mean high water.

### Germination after artificial chilling to break dormancy

Germination of artificially chilled seeds in July and August of 2020 appeared to follow general trends, although most results were not statistically significant (Figure 4). In July, germination rates for *C. lyngbyei* and *J. effusus* generally increased as elevation increased, with the greatest rates at 1.7 m AMSL. By August, seedling mortality had reduced germination rates at all tide heights except for 1.7 m AMSL, where germination had increased. Percent germination of *C. lyngbyei* in August showed a statistically significant difference in mean germination (ANOVA,  $F_{4,15} = 6.6$ ,  $P = 0.003$ ), suggesting a preferred elevation of at least 1.7 m AMSL for recruitment.

*Schoenoplectus tabernaemontani* and *T. latifolia* showed a strong trend in July of germination preference at mid-range elevations, with *T. latifolia* more strongly demonstrating this trend. By August, seedling mortality had largely removed this trend, although *T. latifolia* germination survival remained the greatest at 1.1 m AMSL (mean = 20%). Although trends for these species were not statistically significant, these results suggest a relationship that should be further explored.



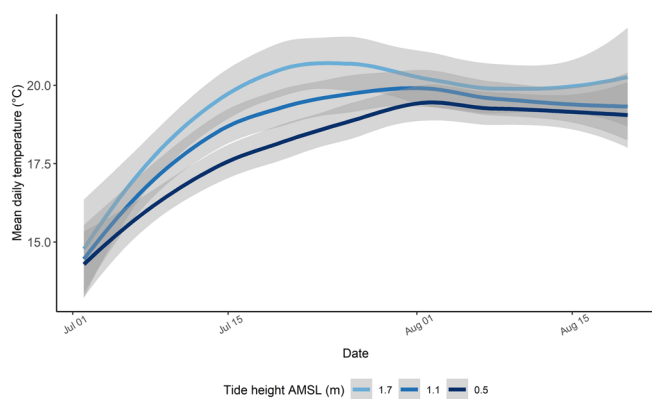
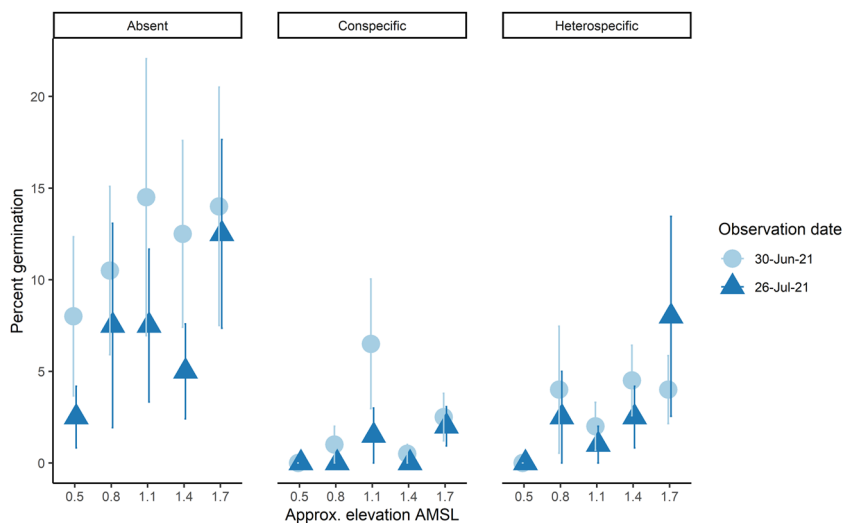
**FIGURE 4** Germination rates of four plant species at five approximate elevations AMSL following artificial chilling (July and August 2020) and natural chilling (May 2021). The same individuals from 20 seeds planted in June 2020 were counted in July and August 2020. Newly planted seeds in the fall of 2020 were observed in May 2021. Points are means  $\pm$  SE ( $N = 4$ ); note the  $y$ -axis showing percent germination is rescaled for each species. Species observed are *Schoenoplectus tabernaemontani* (bulrush), *Typha latifolia* (cattail), *Juncus effusus* (common rush), and *Carex lyngbyei* (Lyngbye's sedge).

### Germination under competitive stress

Germination of *S. tabernaemontani* and *C. lyngbyei* seeds was tested under different conditions of competition; however, *S. tabernaemontani* was the only seed species that germinated during trials with competition

(Figure 5). There was a strong significance for different competitive conditions after controlling for elevation AMSL (ANCOVA,  $F = 22.779$ ,  $P < 0.001$ ). Post-hoc analysis using Tukey's HSD showed *S. tabernaemontani* germination in the absence of competition was significantly greater than when germinating with a conspecific

**FIGURE 5** Germination rates of *Schoenoplectus tabernaemontani* (bulrush) at five approximate elevations AMSL under three different competitive pressures. Seeds exposed to 8-week artificial chilling were planted in mid-May 2021, and the same individuals were observed in late June and late July 2021. Points are means  $\pm$  SE ( $N = 4$ ).



**FIGURE 6** Mean daily temperatures (with 95% confidence bands) logged hourly at the growing surface in the marsh organ pipes across a range of tidal elevation AMSL, with means smoothed for visual convenience. Temperature loggers at each tide height  $N = 12$ .

( $P < 0.001$ ) or heterospecific ( $P < 0.001$ ) transplant, but the species of the competitive interaction was not significant.

### Marsh organ temperature variation

Hourly temperatures recorded were 6–48°C during July and August 2020, although daily temperature fluctuations ranged  $\sim 20^\circ\text{C}$ , and daily temperature means were typically between 18–20°C (Figure 6). A two-way ANOVA of tidal elevation and pipe position within the marsh organ only showed significant differences in daily mean temperatures between tidal elevations ( $F = 19.42$ ,  $P < 0.001$ ), but no significant differences between pipe position, or interaction between tidal elevation and pipe position. Bonferroni-corrected post-hoc analysis showed significant temperature differences only between the highest and lowest tidal elevations at all pipe positions (center  $P = 0.003$ , downstream  $P < 0.001$ , and upstream  $P = 0.007$ ).

## DISCUSSION

Marsh organs have most often been used to conduct transplant experiments, but in this study, I have demonstrated they can also be effective in testing recruitment from seed. Observations of germination following artificial (July–August 2020) and natural (May 2021) chilling cycles suggest that either method of cold stratification for field experiments in marsh organs is reliable; however, success of germination trials may depend on the seasonal temperatures required for the species' germination. Only the results for *C. lyngbyei* demonstrated significant support for my initial hypothesis that seed recruitment would be greatest at the highest elevations, which generates a new hypothesis that clonal growth may be necessary for population expansion. Trends emerged for some species, although not statistically significant, such as greater germination at elevations around the MHW level for *T. latifolia* and *S. tabernaemontani*. Testing for facilitative or competitive interactions showed that *S. tabernaemontani* seeds germinate best in the absence of a neighbor plant. However, germination was not entirely suppressed when paired with a neighbor plant, especially as inundation stress is relieved at higher elevations.

*Carex lyngbyei* had  $\sim 50\%$  higher germination rates under natural overwinter chilling compared to artificial chilling, although not significantly different, and were comparable to lab trials under similar chilling and salinity manipulations (Maguire and Heuterman, 1978). Overall, seeds of *C. lyngbyei* germinated best above the MHHW level (1.7 m AMSL). Although germination did occur at the MHW level (1.4 m AMSL), the significantly lower germination rates and mortality suggest that ideal recruitment elevations are above monthly tidal intrusion, and that the species predominantly spreads clonally to lower elevations.

In contrast, species that occupy lower elevations in the marsh elevation profile or tolerate more saturated conditions, such as *S. tabernaemontani*, may germinate better around the MHW level. Although observed germination

rates for *S. tabernaemontani* in May 2021 following overwinter chilling were comparable to those in lab studies (Rosbakh et al., 2019), nearly significant germination rates at elevations around the MHW level suggest the ideal recruitment elevation may be lower in the marsh profile where more regularly saturated conditions support germination. Marsh organ substrate at the highest elevation appeared to dry out over the summer of 2020, likely due to evaporation at the growing surface occurring faster than water could be wicked through the sediment. The seedling mortality for *S. tabernaemontani* at elevations above the MHW level in August 2020 indicates that even though conditions were initially sufficient to germinate, the substrate dried out too much between inundation events to support the young seedlings.

In July 2020, *T. latifolia* seeds appeared to have a trend of germinating favorably at the MHW level, but had suffered mortality by August. Although seeds of many emergent wetland species can germinate when submerged in water for short periods of time, they may suffer mortality if submergence is prolonged (Boedeltje et al., 2002). Despite drain holes in the pipes allowing excess water to drain, most pipes at or below the MHHW level retained some standing water (~1 cm). *Juncus effusus* did not germinate well in any trials, although germination rates for artificially chilled seeds at the highest test elevation in August 2020 were comparable to germination rates under lab conditions (Maguire and Heuterman, 1978). This may indicate that the ideal recruitment niche for *T. latifolia* and *J. effusus* is above the tidal profile where soils are saturated by water movement through the soil.

Whether seed germination is facilitated by a neighbor plant can be highly specific to the species of mature neighboring plants (Ohsaki et al., 2020), and may change with density dependence (Pranchai et al., 2018). Although *S. tabernaemontani* seeds germinated significantly better in the absence of a mature neighbor, they were not entirely inhibited from germinating when paired with a near-neighbor competitor. Germination rates of *S. tabernaemontani* seeds were not significantly affected by the species of their mature neighbor, although a slight trend of greater germination and greater survival when paired with a heterospecific species (*C. lyngbyei*) may warrant further investigation to test whether species-specific interactions help with seed recruitment under competitive and environmental stressors, such as oxygen release from the rhizosphere (Brix, 1994). Whenever native seeds are used in conjunction with transplants in restoration projects, it is important to understand what competitive pressures exist on germination, and what spacing between transplanted individuals is sufficient to reduce competitive interactions on seed recruitment while promoting facilitation of abiotic conditions.

Differences in marsh organ temperatures recorded at different heights were largely due to the tide cycles that flush the marsh organs with cooler water. This resulted in the lowest test heights (0.5 m AMSL) being cooler than the highest test heights, because the lowest levels were flooded for several hours daily regardless of the timing of the tide

cycle. Although this introduces some variation to the design, this also captures natural temperature changes along elevation gradients. Mean temperatures observed at all tide heights in the marsh organs were within ranges suitable for germination of the species tested (Schütz and Rave, 1999) and are not suspected as an explanation for lack of germination. Additionally, the range of temperatures observed in the marsh organs is comparable to temperatures observed on bare mudflats at a similar latitude (Guarini et al., 1997), and may be considered representative of what seeds might experience at an unvegetated site. Because seed germination is temperature sensitive, future experiments that focus on timing of phenological events should control for temperature differences across an elevation gradient.

## Applications

Marsh organs can be an effective experimental method to understand the recruitment potential of seeds across marsh profile elevations, with applications to restoration or conservation. This experimental design could be used to test the effect on germination of interactions between elevation and other environmental variables, such as nutrient levels or boat wave action. Additionally, future studies may employ marsh organs to investigate recruitment of invasive species without as great a risk of spreading seed into the native community. Tests of native or non-native seedling survival can inform understanding of community succession, although experiments may require a larger pipe diameter to accommodate effects of competition. Experimentally testing early plant life stages in as near-natural conditions as possible can generate new inferences about habitat replacement and turnover through species immigration and seed bank dynamics. Additionally, seeds are an attractive option in restoration projects because they are more cost-effective and easier to disperse than transplanting; however, their establishment success is hard to estimate and even harder to guarantee (Baldwin, 2004; Zhu et al., 2014; Löhmus et al., 2020). Marsh organs provide a method to test species-specific germination responses with opportunities to manipulate environmental variables and biotic interactions. The resulting inferences can be used to inform restoration design and anticipate conservation management needs as plant community composition shifts due to environmental variability like sea level rise. Estuaries of the Pacific Northwest are generally geographically restricted by steep and rocky hillslopes, which will limit vertical marsh migration as sea levels rise (Thorne et al., 2018). Forecasting compositional and distribution shifts of TFM and tidal marsh plant communities under different sea level rise scenarios is of increasing importance to land managers, especially to quantify the effects of reduced habitat on salmonid population dynamics (Davis et al., 2021). Identifying where to anticipate preferential



recruitment niches for key community-forming species will help land managers plan to accommodate habitat transitions.

## AUTHOR CONTRIBUTIONS

S.L.L. confirms sole responsibility for study conception and design, data collection, analysis and interpretation of results, and manuscript preparation and final approval.

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## OPEN DATA BADGE/OPEN MATERIAL BADGE



This article has been awarded Open Data and Open Materials badges. All materials are publicly accessible via the Open Science Framework at <https://github.com/stefanielane/MarshOrgans> and <https://doi.org/10.5061/dryad.gmsbcc2qw>. Learn more about the Open Practices badges from the Center for Open Science: <https://osf.io/tyvxyz/wiki>.

## DATA AVAILABILITY STATEMENT

Scripts and data for all analyses in this contribution are available at <https://github.com/stefanielane/MarshOrgans>, or available on Dryad at <https://doi.org/10.5061/dryad.gmsbcc2qw>.

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