



Effects of salinity, temperature, and immersion conditions on seed germination of invasive *Spartina alterniflora* Loisel (smooth cordgrass) in Japan

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ABSTRACT

Spartina alterniflora is a halophytic perennial grass species that has invaded coastal wetlands worldwide, including tidal flats in Japan. Rapid removal or eradication of invasive plants is desirable because of their severe ecological impact on local ecosystems. Seed germination is the main cause of its distribution and invasion. Understanding germination characteristics of *S. alterniflora* is critical for the prediction and early detection of its populations in new environments. Therefore, we aimed to investigate the effects of salinity (0, 10, 20, 30, and 40 g/L artificial seawater), temperature (15, 20, 25 °C for constant, and 15/25 °C for alternating), and seed immersion (5 ml and 40 ml medium) based on laboratory experiments to evaluate the physicochemical environmental factors that facilitate the germination of *S. alterniflora* seeds. The cumulative germination rate and mean germination time were significantly affected by temperature and salinity but not by immersion. Increased salinity gradually reduced the cumulative germination rate in all temperature and immersion conditions. The mean germination time tended to be prolonged with the salinity level. A constant temperature of 25 °C promoted higher seed germination compared to constant temperatures of 15 and 20 °C. Notably, alternating temperature between 15 and 25 °C induced a higher seed germination compared to other constant temperatures. In addition, alternating temperatures varied the mean germination time to facilitate sensitivity to salinity, indicating that temperature fluctuation is a key factor that stimulates seed germination of *S. alterniflora*. These results suggest that *S. alterniflora* seeds prefer gaps in vegetation canopies and a low depth of burial in soil. Further studies are needed to analyze prevention strategies for the eradication of this invasive species in Japan.

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1. Introduction

Spartina alterniflora Loisel (smooth cordgrass) is a perennial salt marsh grass native to the Atlantic and Gulf Coast of North America (Biber and Caldwell, 2008). The ability of this species to trap large volumes of tidal sediments has led to its deliberate introduction in several parts of the world to accelerate natural land formation and protect the shoreline from erosion (Bertness, 1991; Chung, 2006; Li et al., 2018; Qi et al., 2017; Xue et al., 2018). In contrast, *S. alterniflora* replaces many native species and becomes the dominant species in the local ecosystem of salt marshes of the intertidal zone because of its stout stems and strong roots, which are resistant to salt and sea flooding and hence, it has a high reproductive capacity (Zhang et al., 2004; Du et al., 2019). To date,

S. alterniflora has invaded and expanded its distribution in salt marshes and tidal flats in many regions of the world, including the west coast of the United States (Spicher and Josselyn, 1985; Callaway and Josselyn, 1992), Northern Europe (Baumel et al., 2003), South Africa (Adams et al., 2012), New Zealand (Partridge, 1987), China (Zhang et al., 2017), and Japan (Kimura et al., 2016). The rapid expansion of this plant is now considered a threat to coastal environments (Liu et al., 2017). For instance, the invasion of *S. alterniflora* often alters the structure of trophic functional groups of nematodes, macrobenthic invertebrates, and migratory waterbird communities (Li et al., 2009; Gan et al., 2009; Zou et al., 2016). Thus, the management of the non-native *S. alterniflora* is required in the regions of invasion (Hedge et al., 2003; Tang et al., 2018).

In Japan, *S. alterniflora* was first detected in Aichi Prefecture in 2008 and then in Kumamoto Prefecture in 2009, followed by its identification in multiple rivers and tidal flats in both prefectures

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(Kimura et al., 2016; Matsuda et al., 2021; Tamaoki and Takizaki, 2018). More recently, new populations were found in the Yamaguchi Prefecture in 2020. Maebara et al. (2020) compared microsatellite sequences of *S. alterniflora* collected from three regions of Japan: Aichi (Umeda River), northern Kumamoto (Tsuboi River), and southern Kumamoto (Ohno River), showing that these three populations were identical to Chinese populations but genetically separated, implying that the three Japanese *S. alterniflora* populations were introduced from China independently. Given the increase in shipping trade between Japan and China, the risk of *S. alterniflora* invasion of the Japanese coastal environment will increase in the future (Maebara et al., 2020).

To conserve the native ecosystem, the eradication of *S. alterniflora* has been attempted in Japan. The dispersal of *S. alterniflora* in Aichi and northern Kumamoto was successfully eliminated with heavy equipment by the local government and citizen groups. However, the vegetation area of *S. alterniflora* has remained and increased in southern Kumamoto for a decade owing to the difficulty of extermination caused by topographical restrictions such as small river width, low amount of water flow, and soft sediment that are posed by the Ohno River, which obstruct the entry of dredging machines into the estuary (Matsuda et al., 2021). The government is conducting a large-scale eradication project by covering the colony with weed-prevention sheets. However, such a large-scale approach is labor- and cost-intensive and may be harmful to native ecosystems. For the eradication of *S. alterniflora* at low and medium budgets, the removal of low-density plants is a better option rather than targeting high-density areas (Taylor and Hastings, 2004). An efficient and cost-effective approach to managing invasive species in the local environment involves early detection and rapid response strategies (Kaiser and Burnett, 2010; Reaser et al., 2020).

Seed germination is the earliest step in a plant's life history (Bewley, 1997). Since the plant grows at the place where the seed germinates, the choice of habitat essentially determines whether the plant will grow successfully (Kitajima and Fenner, 2000). Because of the heterogeneity of habitat and environmental gradients, the position of the seed in the soil influences the probability of its germination and successful emergence, and the subsequent spatial pattern of the population (Grundy et al., 2003). Thus, understanding germination characteristics in response to abiotic factors will facilitate the prediction of the timing and place in which a new individual emerges (Elsey-Quirk et al., 2009; Lamichhane et al., 2019).

Salinity, temperature, and inundation have been effective research targets for regulating the biological performance and ecological distribution of *S. alterniflora* and related *Spartina* species since they often inhabit the intertidal salt marsh (Pennings et al., 2005; Silvestri et al., 2005; Shi and Bao, 2007; Hessini et al., 2009; Xue et al., 2018). Salinity stress is of particular interest to researchers because the community of *S. alterniflora* expands more rapidly in the lower tidal ecological niche than in communities that contain native vegetation (Zhang et al., 2004). Although *S. alterniflora* has a higher tolerance to salinity than other halophytic plants, its germination rate was observed to be repressed in experiments on seed germination with a high salinity condition, suggesting that *S. alterniflora* suffers from salinity stress in typical habitats (Yuan and Shi, 2009; Li et al., 2010; Xiao et al., 2016). In addition to that of salinity stress, the effect of temperature, cold stratification period, light, pH, sulfide concentration, and oxygen deficiency on the performance of seed germination of *S. alterniflora* has been determined (Mooring et al., 1971; Yuan and Shi, 2009; Li et al., 2010; Xiao et al., 2016; Hayasaka et al., 2020). Nevertheless, those studies have not explained why *S. alterniflora* prefers colonization in downstream to upstream in the river likely against the salinity gradient. Thus, it is expected that

Table 1

The monthly climate data in Kumamoto city.

Month	Precipitation (mm)	Daily average temperature (°C)	Maximum temperature (°C)	Minimum temperature (°C)
January	57.2	6.0	10.7	1.6
February	83.2	7.4	12.4	2.6
March	124.8	10.9	16.1	5.9
April	144.9	15.8	21.4	10.6
May	160.9	20.5	26.0	15.6
June	448.5	23.7	28.1	20.2
July	386.8	27.5	31.8	24.2
August	195.4	28.4	33.3	24.8
September	172.6	25.2	30.1	21.2
October	87.1	19.6	25.0	14.9
November	84.4	13.5	18.8	8.8
December	61.2	8.0	12.9	3.4

germination is facilitated by other factors rather than salinity. To answer the question, the examination of the influence of multiple abiotic factors on germination characteristics is important. Therefore, we investigated the interactive effects of three abiotic factors including salinity, temperature, and immersion conditions on germination in this study. We hypothesized that germination performance would be repressed with an increase in salinity but would be better by temperature stimulation.

2. Materials and methods

2.1. Seed collection site

Seed collection of *S. alterniflora* was carried out in the Ohno River of Uki City, Kumamoto Prefecture, Japan (32°37'53.8"N and 130°39'35.7"E), which is the same location from where Hayasaka et al. (2020) collected seeds (Fig. 1). The previous survey showed about 21.6‰ salinity around the sampling point and the salinity was gradually decreased along with going to upstream (9.0‰ at 4 km upstream point) (Matsuda et al., 2021). The climate data in Kumamoto city (about 20 km north from the Ohno river) was obtained from Japan Meteorological Agency website, (<https://www.jma.go.jp/jma/index.html>). The annual temperature and precipitation (1991–2020) are 17.2 °C and 2007 mm. The monthly climate data (air temperature and precipitation) is shown in Table 1.

2.2. Seed preparation

We collected ready-to-drop seeds by gentle hand-shattering in early December 2020 to obtain fully mature seeds. The seeds, which were immediately packed in plastic bags with distilled water to prevent water loss, were brought back to the laboratory. Then, the filled seeds were separated from the empty ones using tweezers. The seeds were stored in a plastic bag covered with aluminum foil and filled with distilled water at 4 °C until use for experiments.

2.3. Seed germination experiment

Seed germination was tested under cross combinations of the three factors in a total of 40 treatments comprising of five salinity, four temperature, and two moisture conditions. As seed germination of *S. alterniflora* requires a period of cold stratification for release from dormancy (Biber and Caldwell, 2008), the seeds were kept at 4 °C for 2 months. Filled seeds (50 seeds each) were sown on filter paper (ADVANTEC Filter Paper No. 2, ADVANTEC Co., Ltd, Tokyo Japan) in 90 mm Petri dishes. Saline solutions were prepared at five salinity concentrations of 0, 10, 20, 30, and 40 g/L

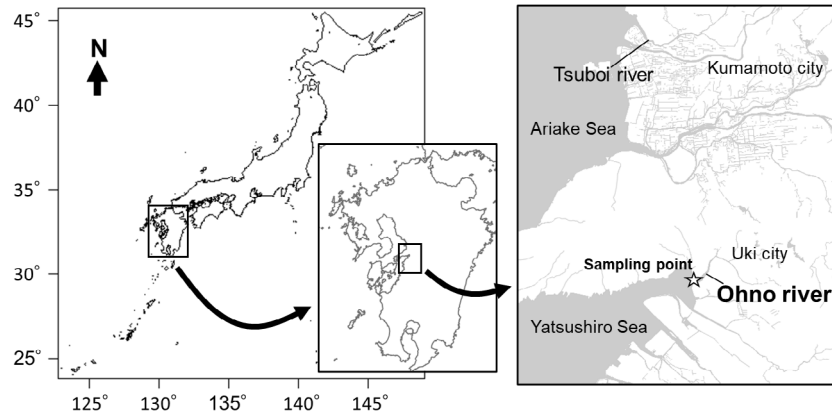


Fig. 1. Location map of sampling field of *Spartina alterniflora* seeds.

Table 2

Summary of conditions examined in this study.

Factors	Conditions
Salinity	0, 10, 20, 30 g/L, 40 g/L artificial seawater powder
Temperature	Constant: 15, 20, 25 °C in 24 h, Alternating: 15/25 °C in 12/2 h
Immersion	Moistened: 5 ml, Immersed: 40 ml

by dissolving artificial seawater powder (SEALIFE, Nihon Kaisui Co., Ltd, Tokyo, Japan) in distilled water. Seeds were moistened with 5 mL or immersed in 40 mL of artificial seawater. The dishes were placed in a temperature-controlled incubator (LH-350S, Nippon Medical & Chemical Instruments Co., Ltd, Osaka, Japan) at three constant temperature conditions (15, 20, and 25 °C) and alternating temperature conditions (15 °C/25 °C for 12 h/12 h) without light irradiation and incubated for 30 days. These three temperatures are close to the daily average temperatures of April, May, and June (Table 1) when *S. alterniflora* seedlings germinated from seeds are observed in the Ohno river. The dishes were removed from the incubator every other day for counting the number of germinated seeds (i.e., those that had ruptured and produced a radicle) in the experiment room at 23 °C, and then, we removed the germinated seeds from the dishes. Considering the effects of temperature change and light irradiation, counting was performed within 30 min. To prevent an increase in the salinity concentration by evaporation, the solution was exchanged every week, and the concentration was checked using a salt meter (PAL-SALT, Atago Co., Ltd, Tokyo, Japan). The examined conditions were summarized in Table 2.

In this study, we calculated the cumulative germination rate (%) and mean germination time (d) using the following formulas (Hayasaka et al., 2020; Liu and Zhang, 2021)

$$\text{Cumulative germination rate (\%)} = \sum_{i=1}^n \frac{Ni}{S} \times 100$$

$$\text{Mean germination time} = \frac{\sum_{i=1}^k \frac{NiTi}{\sum_{i=1}^k Ni}}{\sum_{i=1}^k Ni}$$

where S is the total number of seeds tested in each treatment (50 seeds in this study), Ni is the number of seeds germinated on day i, Ti is the number of days from the start of the experiment to the ith observation day, n is the time of the germination test (30 days in this study), and k is the number of days.

2.4. Data analysis

Statistical analyses were performed according to Hayasaka et al. (2020). We used a generalized linear model (GLM) with

a binominal distribution and a long link function to test the effect of salinity, temperature, and immersion on the cumulative germination rate. The cumulative germination rate was used as a response variable, and salinity level (0, 10, 20, 30, and 40 g/L), temperature (15, 20, and 25 °C, and alternating temperature), immersion (moistened versus immersed), and their interactions were used as explanatory variables. The effects of these factors on mean germination time were analyzed using a linear mixed model (LMM). The in-transformed mean germination time of each seed was used as a response variable and the three factors and their interactions were included as explanatory variables. Dish identification was included in LMM as a random factor. The significances of explanatory variables of GLM and LMM were tested using type-II likelihood ratio and type-II Wald F tests with the Kenward–Roger approximate denominator degree of freedom, respectively. A P-value less than 0.05 was considered statistical significance. These tests were followed by Tukey pairwise comparisons. These analyses were performed using the statistical software R (version 4.1.1; R Core Team, 2017).

3. Results

Seed germination of *S. alterniflora* occurred in all treatments. The germination curves exhibited different patterns that were affected by salinity, temperature, and moisture conditions (Fig. 2). The maximum and minimum cumulative germination rates were $89.5 \pm 6.6\%$ under alternating temperature with moistened treatment using 0 g/L saline water and $14.5 \pm 3.4\%$ under 15 °C constant temperature with moistened treatment using 40 g/L saline water, respectively (Figs. 2 and 3). Under the constant temperature condition, the germination rate increased with temperature: 15%–40% cumulative germination rate at 15 °C, 16%–54% cumulative germination rate at 20 °C, and 22%–68% at 25 °C (Fig. 3). However, alternating temperature treatment remarkably increased cumulative germination rate (46%–90%), which was more than any other constant temperature condition (Figs. 2 and 3). Besides temperature, salinity also influenced germination rate. While seeds treated with distilled water (0 g/L salinity) exhibited 39%–90% cumulative germination rate, increase in salinity resulted in a gradual reduction in the germination rate: 32%–82% (10 g/L salinity), 30%–82% (20 g/L salinity), 19%–67% (30 g/L salinity), and 15%–55% (40 g/L salinity) (Figs. 2 and 3). The effect of inundation treatment was unclear. The cumulative germination rates were 15%–90% and 19%–82% in moistened and immersed treatments, respectively. Statistical analysis showed significant effects of temperature ($p < 0.001$) and salinity ($p = 0.031$), but no significant difference was observed between moistened and immersed conditions ($p = 0.863$, see Table 3). In addition, the

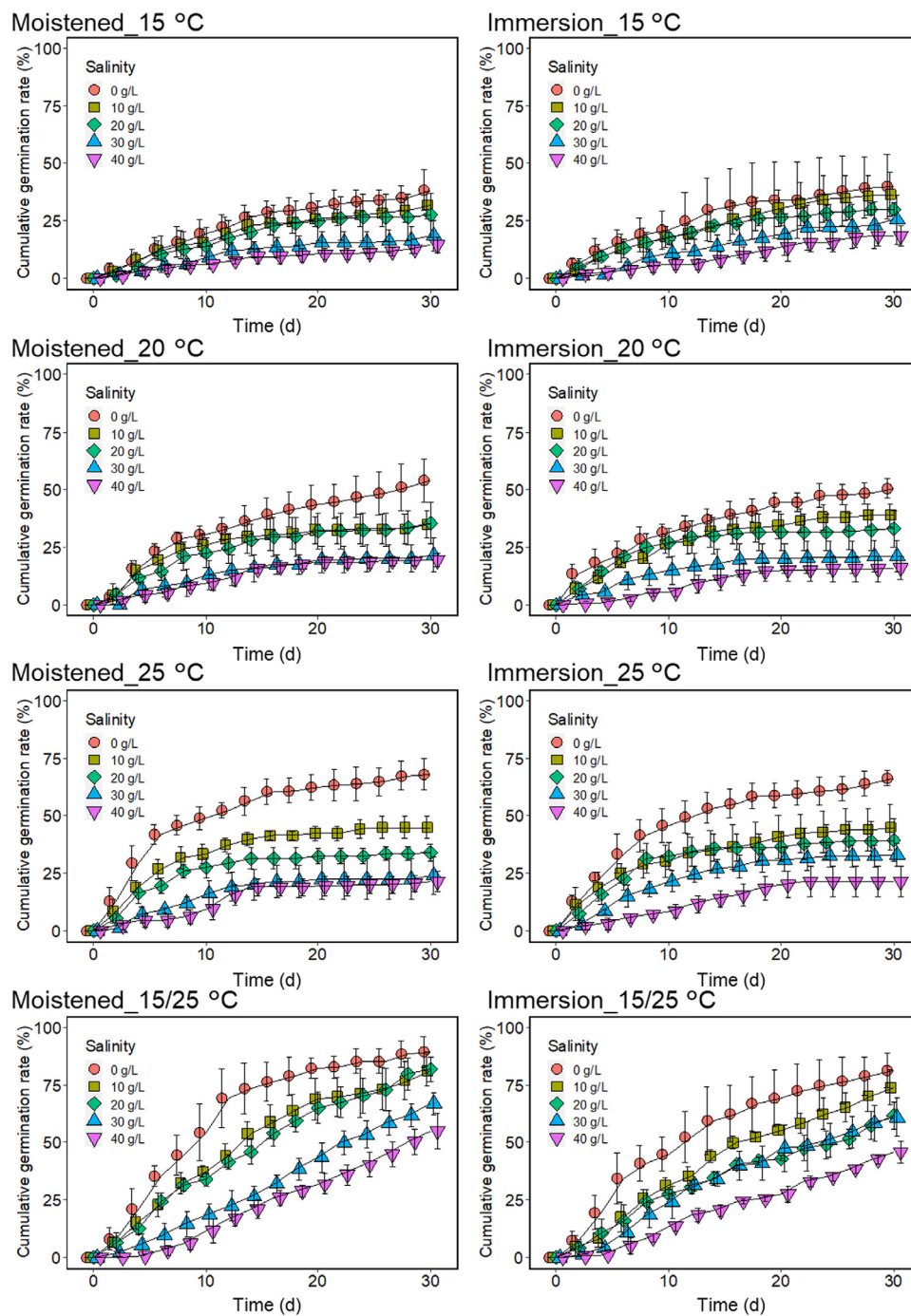


Fig. 2. Germination curves (%) of *S. alterniflora* seeds. Seeds were incubated with 5 mL (moistened) or 40 mL (immersed) saline water (0, 10, 20, 30, and 40 g/L) in different temperature conditions (15, 20, 25 °C, and 15/25 °C alternating for 12 h each). Fifty seeds were sown on a dish after a cold stratification period of two months and incubated for 30 days. Values are the mean ($n = 4$) \pm standard deviation. Symbols are as follows for salinity concentrations: circle: 0 g/L, square: 10 g/L, diamond: 20 g/L, triangle: 30 g/L, and inverted triangle: 40 g/L.

interaction terms between temperature, salinity, and moisture conditions were not significant (Table 3).

Under constant temperature conditions, the mean germination time was prolonged by salinity and shortened by an increase in temperature (Fig. 4). Under alternating temperature conditions, the mean germination time varied with salinity (Fig. 4). Immersion had no significant effect (Fig. 4). These trends are consistent with the cumulative germination rates with marginal differences (Fig. 3). The mean germination time was delayed by alternating temperature compared to that of constant temperature. Furthermore, under constant temperature, the mean germination time

was shortened in the cases where salinity was 10 or 20 g/L. Germination occurred in the early days after the beginning of the experiment under constant temperature conditions because of shorter germination time, and this activity was weakened in the latter phase. The maximum and minimum germination times were 18.3 ± 1.6 days under alternating temperature with moistened treatment using 40 g/L saline water and 7.2 ± 1.0 days under 20 °C constant temperature with moistened treatment using 20 g/L saline water, respectively. Statistical analysis showed significant effects in terms of salinity, temperature, and interaction between salinity and temperature (each $p < 0.001$, see Table 3).

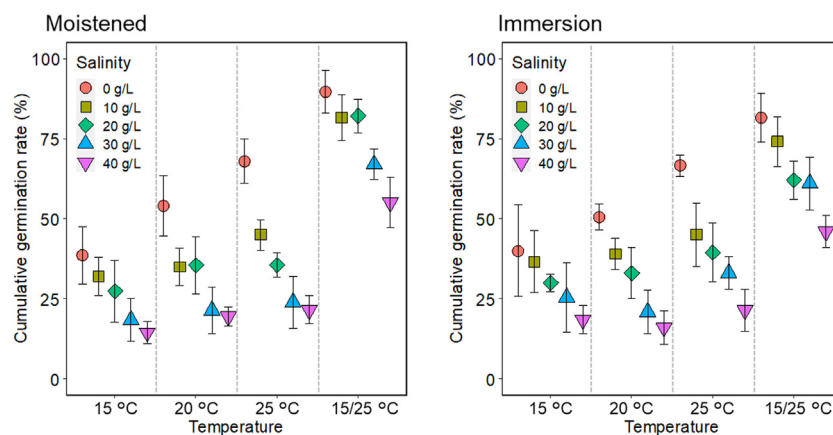


Fig. 3. Cumulative germination rate (%) among the combination of the three conditions (salinity, temperature, and immersion) after incubation of *S. alterniflora* seeds for 30 days. The left and right boxes indicate the immersion treatment of seeds moistened with 5 mL solution and seeds immersed in 40 mL solution, respectively. Values are expressed as the mean \pm standard deviation ($n = 4$). Symbols are as follows for salinity concentrations: circle: 0 g/L, square: 10 g/L, diamond: 20 g/L, triangle: 30 g/L, and inverted triangle: 40 g/L.

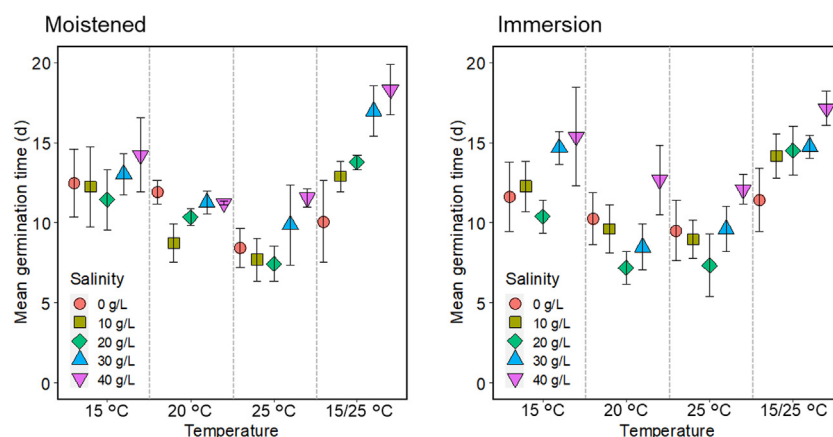


Fig. 4. Mean germination time (d) among the combination of conditions of the three factors (salinity, temperature, and immersion) after incubation of *S. alterniflora* seeds for 30 days. The left and right boxes indicate the immersion treatment of seeds moistened with 5 mL solution and seeds immersed in 40 mL solution, respectively. Values are expressed as the mean \pm standard deviation ($n = 4$). Symbols are as follows for salinity concentrations: circle: 0 g/L, square: 10 g/L, diamond: 20 g/L, triangle: 30 g/L, and inverted triangle: 40 g/L.

Table 3

Effects of salinity, temperature, and immersion on the germination performance of *S. alterniflora* seeds. Cumulative germination rate and mean germination time were analyzed using GLM and LMM, respectively. Bold characters indicate statistical significance ($P < 0.05$).

Explanatory variable	Cumulative germination rate			Germination time		
	df	LR- χ^2	P	df	F	P
Salinity (S)	4	10.6179	0.0312	217.54	25.0214	<0.001
Temperature (T)	3	17.6019	<0.001	216.3	80.6888	<0.001
Immersion (I)	1	0.0298	0.863	267.77	0.5748	0.449
S \times T	12	0.5185	0.999	180.4	3.3352	<0.001
S \times I	4	0.1346	0.997	217.54	1.9398	0.105
T \times I	3	0.5981	0.897	216.3	1.0561	0.368
S \times T \times I	12	0.1504	1	180.4	1.4266	0.157

4. Discussion

In this study, we tested the combined effects of three abiotic factors, salinity, temperature, and immersion, on seed germination of *S. alterniflora* in laboratory conditions. Higher salinity suppressed seed germination, whereas a higher constant temperature increased the germination rate, but immersion had no significant effect (Fig. 3). Furthermore, a higher germination rate was recorded at an alternating temperature than at a constant temperature (Fig. 3). Interestingly, the alternating temperature with 40 g/L seawater resulted in $55 \pm 3.8\%$ (moistened) and $46 \pm 5.7\%$ (immersed) cumulative germination rate. These values were comparable to $54 \pm 7.7\%$ (moistened) and $50.5 \pm 12.2\%$ (immersed) under constant temperature (20 °C) with 0 g/L salinity. This indicates that alternating temperature is an important factor to facilitate seed germination in high salinity conditions.

The highest germination rate achieved in this study was approximately 90% (Fig. 3) in contrast to the germination rate of 65%–70% reported by Hayasaka et al. (2020). This difference was probably caused by the difference in the time of collection of seeds (December by us and November by them) as the spikelets were persistently attached to the spikes in their seeds indicating

that they were not adequately mature in November 2020. Similar to our study, Li et al. (2010) collected seeds after maturation (ready-to-drop stage) in Tianjin, China, and showed a maximum germination rate of 90% without cold stratification. According to Biber and Caldwell (2008), in Mississippi, USA, seeds collected prior to full maturation exhibit a decrease in the rate of germination, while seeds that have already dropped off cannot be collected past the best timing; therefore, collection should be performed within seven days after seed production. In the case of the Ohno River, most of the seeds had dropped off by January, indicating that the one-month time lag affected the rate of germination in the experiment compared to Hayasaka et al. (2020).

The rate of germination decreased across the salinity gradient (Fig. 2). Previous studies examining the effect of salinity on *S. alterniflora* seed germination used NaCl as a salinity adjustment (Yuan and Shi, 2009; Li et al., 2010; Xiao et al., 2016). Since 100 mM NaCl of medium contains 5.85 g/L salt powder, 10, 20, 30, and 40 g/L of salinity are regarded as equivalent to approximately 170, 340, 510, and 680 mM NaCl respectively. The detrimental effect of salinity levels in this study was comparable to those studies (Yuan and Shi, 2009; Li et al., 2010; Xiao et al., 2016). Salinity is a common constraint for plant survival, and *S. alterniflora* seeds are likely to suffer from salt stress in their habitats (Isayenkov and Maathuis, 2019; Akyol et al., 2020). Nevertheless, *S. alterniflora* grows vigorously in brackish and saline waters (Biber and Caldwell, 2008). Some experiments indicated that low salinity levels (100–200 mM NaCl) marginally promoted the growth of *S. alterniflora* and that lower or higher salt levels inhibited its growth (Lewis and Weber, 2002; Ma et al., 2011), suggesting that a physiological difference in the sensitivity to salt between seeds and seedlings exists because the salt glands of *S. alterniflora* are capable of secreting approximately half of the ions to help in adjusting to the changing osmotic environment (Bradley and Morris, 1991). Thus, there are advantages to growing up to seedlings in response to salt stress. Considering that the mean germination time was shortened at low salinity levels (10–20 g/L) under constant temperature conditions (Fig. 4), rapid germination under low salinity conditions is due to the development of seedlings for rapid adaptation to salinity.

Under constant conditions, the seeds exhibited an increase in the rate of germination and a shortening of the mean germination time as the temperature increased (Figs. 2 and 3). The *S. alterniflora* population in the Ohno River was estimated to have invaded through China, and the plant populations in China were originally introduced from the Gulf of Mexico, USA (Maebara et al., 2020; Liu and Zhang, 2021). The preference for warm temperatures in seed germination may reflect the southern sources of the origin of *S. alterniflora*. However, the alternating temperature induced a higher rate of germination than the warm constant temperature (25 °C) (Fig. 3). In addition to the rate of germination, germination time was more sensitive to salt stress under alternating temperature conditions (Fig. 4). The marked change in seed germination performance induced by alternating temperatures indicates that diurnal temperature fluctuation is an important factor for the invasion of *S. alterniflora* into new habitats.

The effect of alternating temperature on seed germination has been observed in many plant species (Kato and Kadono, 2011; Christian and Goggi, 2012; Kumar et al., 2013), and it has also been confirmed in *S. alterniflora* (Mooring et al., 1971; Hayasaka et al., 2020). Daily temperature fluctuations usually occur at or near the soil surface in open habitats, and the amplitude is alleviated below the plant canopy, underwater, and with increasing depth of burial in soil (Akkuzu et al., 2010; Singh and Sharma, 2017). The temperature in intertidal fields also changes daily owing to tidal fluctuations in addition to air temperature variation (Harrison and Phizacklea, 1987; Jacobs et al., 2008; Kim

et al., 2010). Given that field temperature fluctuation alleviates the amplitude of temperature during immersion, which has no significant effects on seed germination in nature (Table 3), tidal fluctuation is a potent factor that induces seed germination. Thus, intertidal and supralittoral zones are optimal conditions for *S. alterniflora* seed germination. This germination feature is consistent with the habitat prediction model that showed that the altitude above sea level was the main factor responsible for the invasion of *S. alterniflora* in Japan (Masuda et al., 2021).

S. alterniflora is known to have relatively high tolerance to salinity compared to other halophytic plants (Vasquez et al., 2006; Medeiros et al., 2013). Since the salt content in the soil usually depends on the elevation within the intertidal zone, the salinity gradient determines the vegetative zonation patterns in salt marshes (Perry and Atkinson, 1997; Emery et al., 2001). *S. alterniflora* often grows and replaces native species in the lower tidal zone, whereas the upstream area is occupied by other halophytes (e.g., *Phragmites australis* and *Juncus roemerianus*) because of the weakness of *S. alterniflora* in competition with other halophytes within the high tidal zone (Pennings et al., 2005; Gan et al., 2009; Yuan et al., 2013). To avoid habitats of neighboring plants, *S. alterniflora* may germinate in the bare field by detecting the amplitude of diurnal temperature that is smaller in the ground below the plant canopy than in open fields (Akkuzu et al., 2010), which explains the reason for its preferential growth in the low tidal zone, although germination is inhibited under high-salt conditions. Conversely, the structure, including the vegetative canopy and artificial weed-prevention sheets that afford shade, will reduce the germination of *S. alterniflora*. In the case of the Ohno River, *S. alterniflora* rarely grew in the upstream region occupied by *P. australis*, although the environmental conditions were likely to be suitable for its invasion (Matsuda et al., 2021). Recently, local and national governments have started an eradication project with weed-prevention sheets in the Ohno River. However, the impact of this method on benthic communities is poorly understood. To prevent *S. alterniflora* invasion in eco-friendly conditions, the management of native vegetation in the river should also be focused on.

This study characterized seed germination performance of *S. alterniflora* against salinity, temperature, and immersion stresses in laboratory conditions, whereas we excluded verifying the effects of other abiotic factors such as pH, light, oxygen deficiency, and cold stratification period. Those environmental factors should be verified in further study. In this study, seeds were incubated without light irradiation in the incubation. Although this dark treatment may play negative effects on germination performance, the maximum germination rate achieved in this study was approximately 90% in the suitable condition (under the alternating temperature without salt) (Fig. 3). This value was comparable to or more than previous studies (Yuan and Shi, 2009; Li et al., 2010; Xiao et al., 2016; Hayasaka et al., 2020; Liu and Zhang, 2021). Thus, this study adequately showed the interactive effect of salinity and temperature. This point is a strength of this study. On the other hand, the other physiological indicators such as germination recovery, antioxidant index, and seedling growth after germination have not been analyzed. In addition, all experiments have been conducted in controlled conditions. To overcome those weaknesses of this study, field experiments will be carried out and further investigation on plant growth and stress tolerance will enhance the controlling strategies for the invasive plant.

5. Conclusions

The present study demonstrated that salinity and temperature independently have significant impacts on the germination success of *S. alterniflora* seeds, whereas immersion had no effect.

Similar to that in other plants, salinity hampered germination, whereas temperature fluctuations remarkably stimulated germination. Data on the combined effect of salinity and temperature imply that *S. alterniflora* preferentially germinates on the surface of the bare field in intertidal or supralittoral zones, even under saline conditions, because vegetative canopy, inundation, and deep burial in soil alleviate the daily temperature amplitude. This study provides ecological insights into the strategies of *S. alterniflora* seeds that preferentially germinate in lower tidal zones. Further practical studies on prevention efficiency are required to evaluate the eradication of *S. alterniflora* from within the invasive range.

CRediT authorship contribution statement

Ryuya Matsuda: Implementation of all experiment, Paper writing. **Katsumasa Yamada:** Statistical analysis. **Daisuke Hayasaka:** Experimental design. **Yasuhisa Henmi:** Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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