

High aqueous salinity does not preclude germination of invasive *Iris pseudacorus* from estuarine populations

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Abstract. Estuarine ecosystems are threatened by climate change and biological invasions. Among global changes, sea-level rise is broadly impacting tidal wetlands, through increases in salinity and alteration of inundation regimes. Extant freshwater plant species are often presumed to be limited to reaches of estuaries with low salinity and narrow tidal ranges. However, the potential for invasive freshwater species (e.g., *Iris pseudacorus*) to persist and spread with increased salinity and flooding is poorly understood and can jeopardize native biodiversity and other wetland ecosystem services. The successful establishment of invasive plants will be dependent on their tolerance to salinity and inundation, starting with the germination life stage. Changes to abiotic estuarine gradients may alter the germination process of tidal wetland plant species that underlies significant patterns of plant community composition and biodiversity. We explored germination responses of seeds from two invasive *I. pseudacorus* populations from freshwater and brackish tidal sites in California's San Francisco Bay–Delta Estuary. We tested germination dynamics under salinity levels ranging from freshwater to seawater (0, 12.5, 25, and 45 dS/m) and two hydrological conditions (moist and flooded). Salinity levels >12.5 dS/m inhibited germination of seeds from both populations, consistent with viviparism and seedling emergence recorded at field sites. However, seeds exposed to seawater for 55 d germinated once exposed to freshwater. Germination velocity and seed buoyancy differed between populations, likely due to differences in seed coat thickness. Our results demonstrate that after 55 d in seawater, buoyant seeds of *I. pseudacorus* retain their ability to germinate, and germinate quickly with freshwater exposure. This suggests that invasive populations of *I. pseudacorus* can colonize new sites following potentially long-distance dispersal of buoyant seeds with tidal currents. These findings inform risk assessments and highlight the need to prioritize the management of invasive *I. pseudacorus* in estuarine ecosystems impacted by rising sea level.

Key words: coastal wetlands; hydrotime models; macrophyte; seaborne seeds; sea-level rise; seed morphology; vivipary.

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INTRODUCTION

Estuaries are sensitive ecosystems threatened by biological invasions and sea-level rise (SLR). Salinity and inundation regimes are significant physical drivers of the distribution and abundance of plant species within tidal wetlands (Contreras-Cruzado et al. 2017). Along with biotic interactions, salinity and inundation influence the response of wetland plant species to SLR along estuarine gradients (Engels and Jensen 2010). In the San Francisco Bay–Delta Estuary, the largest estuary on the Pacific Coast of North America, SLR and resultant increases in salinity are impacting tidal wetlands, yet there remains a need for research on how wetland plant species will respond to combinations of climate change impacts such as increases in both salinity and inundation (Gallego-Tévar et al. 2019, Parker and Boyer 2019).

A wide range of aqueous salinity concentrations are found in estuaries, which affect the establishment of tidal wetland plant species depending on the sensitivity of germination, emergence, and seedling survival to salinity (Muñoz-Rodríguez et al. 2017, Dalziell et al. 2020). Indeed, salinity represents a potential stressor for many organisms due to toxic accumulation of salt in cells and induced changes in osmotic conditions reducing plant water availability (Parihar et al. 2015). Unlike halophytes, glycophytes do not have morphological adaptations and mechanisms to excrete or exclude salts, and exhibit physiological stress responses even at low salt concentrations (Greenway and Munns 1980, Van Zandt et al. 2003). In addition to salinity, tidal inundation may impose high stress levels to estuarine vegetation due to anoxia and long flooding periods (Colmer and Voesenek 2009). Therefore, the abiotic constraints imposed by SLR could drastically limit species survival, growth, and reproduction, influencing the distribution and abundance of less tolerant biota, including that of invasive glycophytes (Eller et al. 2017). The management of invasive species must be grounded in knowledge of their ecology and environmental tolerances, and consideration of the recruitment phase of the life cycle in response to changing environmental conditions is crucial to curb invasions and establish conservation strategies.

The alternative fates of a seed dispersed into an environment include germination, dormancy, mortality, or removal via predation, flow, and other processes (Chambers and MacMahon 1994). As an irreversible process preceding seedling emergence, seed germination is a pivotal stage in plants' life cycle, dictating the environment under which resultant seedlings will grow (Fenner and Thompson 2005). Successful recruitment of many plant species therefore depends on proper germination responses to environmental conditions (Donohue et al. 2010). Increasing salinity can alter the timing and success of germination of tidal wetland species, depending on their sensitivity (Greenwood and MacFarlane 2006, Goodman et al. 2011). When environmental conditions are not favorable for seedling survival, germination can be partially or totally impeded, with seeds remaining dormant or quiescent and germinating once exposed to lower salinity (Muñoz-Rodríguez et al. 2017). This germination recovery depends on the extent and duration of exposure to salt stress that influences the survival of embryos or induces prolonged dormancy to protect seeds (Infante-Izquierdo et al. 2019). Thus, increasing salinity due to SLR will likely modify the germination capacity of some estuarine species (Janousek and Folger 2013) and change the composition, structure, and functions of tidal wetland communities (Nielsen et al. 2008). Germination is also controlled by tidal inundation ensuring seed hydration (Zhao et al. 2020). Knowledge of seed ecology is essential for understanding native and invasive species responses to climate change (Walck et al. 2011) as the breadth of germination niche can determine distributional ranges of species. The most tolerant and competitive species and those presenting high acclimation abilities would likely be most able to take advantage of changing conditions. The fact that such plant species could be nonnative or invasive represents a conservation management concern.

Iris pseudacorus L. (yellow flag iris; Iridaceae), native to Europe, North Africa, and western Asia, has been introduced as an ornamental pond and garden plant in almost all ecozones of the world (Gervazoni et al. 2020). This perennial herb has escaped cultivation and naturalized in many areas, and although it is often regulated as a noxious weed in its exotic range, it continues to

be sold by nurseries and on the Internet for horticultural purposes. As invasive spread of populations of *I. pseudacorus* has recently been increasing, the species has become more of a focus in impacted wetlands. In the United States and Japan, the species shows competitive advantages over native congeners through high biomass production (Mopper et al. 2016), and reduces plant biodiversity, especially that of native species, when present in dense patches (Hayasaka et al. 2018). In addition, population dynamic models projected the exclusion of the native *Iris hexagona* Walter by *I. pseudacorus* in freshwater tidal marshes (Pathikonda et al. 2009). Invasive spread of *I. pseudacorus* was thought to be due to its high clonal reproduction through rhizomes, but Gaskin et al. (2016) showed that sexual reproduction is the primary reproductive strategy, unlike many aquatic plant species. Seeds of *I. pseudacorus* disperse directly from mature capsules to water surface and float due to the presence of an air space under the seed coat. Buoyancy at water surface is maintained for months (Coops and Vandervelde 1995, van den Broek et al. 2005), posing a large risk of seed dispersion by hydrochory, and of potential population establishment in new sites.

Based on observations in the native European range, Sutherland and Walton (1990) refer to *I. pseudacorus* as a glycophyte with limited salt tolerance that grows best in freshwater; yet, they also document changes in morphology of the species across an intertidal elevation gradient and found high rates of seedling recruitment in the low zone of a salt marsh. Given the high genotypic diversity of *I. pseudacorus* invading wetlands in the Pacific Northwestern United States (Gaskin et al. 2016), variation in the sensitivity to salinity and inundation stress of invasive populations may be a factor affecting distribution and spread. *I. pseudacorus* is now spreading from freshwater tidal wetlands in the inland Sacramento—San Joaquin Delta to downstream sites in California's San Francisco Estuary. This expansion raises concerns about the degree to which the species may tolerate the wide range of estuarine salinity and inundation levels, along with global changes such as SLR. The shorelines of the San Francisco Bay—Delta Estuary were historically fringed by expansive tidal wetlands, and Suisun Marsh was the largest brackish wetland in the western United

States (Brown et al. 2016). This region is currently slated for an ambitious restoration plan through removal of water control structures to support the recovery of endangered fauna and flora, native diversity, and tidal wetland ecosystem recovery. As tidal wetland habitat develops, it will be particularly vulnerable to invasion by plant species such as *I. pseudacorus*.

Evaluation of germination responses of invasive populations to environmental factors is of critical importance for understanding post-introduction acclimation or adaptation and for risk assessments and prioritization of management strategies in the context of global changes. Knowledge gaps on seed ecology are high for many species, and this lack of data limits our ability to effectively restore ecosystems and protect threatened plant populations (Ribeiro et al. 2016). As sea level rises and resultant increases in salinity and flooding are impacting tidal wetlands of the San Francisco Bay—Delta Estuary (Parker and Boyer 2019) and elsewhere, there remains a need for research on how wetland plant species will respond to these factors. Our objective was to determine the effects of salinity and water levels on the germination of *I. pseudacorus* seeds from invasive populations at extreme ends of their naturalized range along an estuarine gradient, coupled with field observations and environmental data records. We hypothesized that (1) seed germination fraction will decrease as salinity level increases due to increased seed mortality and dormancy, and (2) germination performance will be superior in moist rather than flooded conditions.

METHODS

Study sites

We investigated germination characteristics of seeds sourced from two populations of invasive *I. pseudacorus* located in intertidal habitats at extreme ends of the species' current estuarine distribution in the Sacramento—San Joaquin River Delta—San Francisco Estuary. The easternmost site, Buckley Cove (BC) at Stockton, California (37°58'38.1" N, 121°22'15.0" W), represents the freshwater end (salinity < 1 dS/m, Appendix S1: Fig. S1). It is furthest upstream along the San Joaquin River, at 141 km hydrologic distance from the Pacific Ocean at Golden Gate Strait

(Appendix S1: Fig. S2). At this site, *I. pseudacorus* has patchily invaded the shorelines, including a central island and small wetland islets, within a protected cove adjacent to the main channel of the river where fringing wetlands include native glycophyte species (Appendix S1: Fig. S3). The westernmost downstream population was at Carquinez Strait (CS) along the Glen Cove shoreline at Vallejo, CA (38°03'54.5" N, 122°12'11.6" W), a 53 km hydrologic distance from the Pacific Ocean (Appendix S1: Fig. S2), at the brackish end of the occupied range (mean salinity ≈ 24 dS/m, Appendix S1: Fig. S1). Here, *I. pseudacorus* was present in a large monospecific patch and in four additional smaller patches. The fringing wetlands include halophytes typical of brackish wetlands, as well as species tolerating both freshwater and brackish conditions near freshwater seeps from steep adjacent hillslopes (Appendix S1: Fig. S3). Aqueous salinity varies through time at this site due to precipitation and freshwater run-off (Appendix S1: Fig. S1). Salinity is highest during mid-summer–fall (30–35 dS/m), when seed maturation and seed dispersal of *I. pseudacorus* take place, and remains high until winter–spring rainfall and Delta outflow freshen the system.

Seed collections

We collected mature seed capsules of *I. pseudacorus* before capsule dehiscence in August–September 2017 from the two study populations. Collections were distributed among 3–5 discrete *I. pseudacorus* patches within populations, with an average of 110 capsules collected per population. Capsules were stored in plastic bags at +4°C immediately after collection.

Seed morphological traits

After transfer from the field, for each capsule, seeds were manually extracted, counted, and weighed at the laboratory. We recorded numbers of seeds with radicle emergence as evidence of precocious germination of seeds within capsules. Next, seeds were air-dried and stored at room temperature in paper bags under dry–dark conditions until a subset of seeds were sampled for use in the experiment.

We measured seed coat thickness using a dissecting microscope (ZEISS Stemi 508; ZEISS Microscopy, Jena, Germany) equipped with a

digital camera. Thirty seeds per population were randomly selected, soaked for 2 d in deionized (DI) water to soften the coat, and bisected crosswise with a razor blade. We photographed thin cross sections under the microscope with 8 \times magnification and measured seed coat thickness at five random points (Image-Pro Insight 9.1 software; Media Cybernetics, Rockville, Maryland, USA).

Greenhouse germination experiment

For each source population, we selected a total of 1,200 fully developed seeds, from 64 capsules from 5 discrete patches of *I. pseudacorus* for BC and from 96 capsules from 3 patches for CS. Selected seeds were exposed to four salinity levels ranging from freshwater to seawater: 0, 12.5, 25, and 45 dS/m. Saline solutions were formulated by diluting synthetic sea salts (Instant Ocean) in DI water to the desired concentration; the 0 dS/m condition was only DI water. Random lots of 25 seeds were placed in lid-covered transparent plastic dishes (6.3 cm height \times 12 cm diameter). Dishes were filled with 3 cm of randomly designated salinity treatments; floating seeds were soaked in solutions for 7 d to ensure hydration. Each dish was then assigned to one of two water level conditions (flooded and moist). Saline solutions were renewed. Seeds in flooded conditions were in 3 cm of solution, while seeds in moist conditions were on moist filter paper placed above a layer of beads (5–6 mm diameter) and imbibed with the assigned saline solution. The 96 dishes (six replicates \times two populations \times four salinity levels \times two water levels) were randomly distributed on benches in greenhouse conditions (supplemental lighting 280 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, photoperiod 12/12 h). Air temperature near the dishes was recorded (HOBO Pro v2 Logger Onset Computer; Bourne, Massachusetts, USA). Salinity and water level were monitored and adjusted during the duration of the experiment. We monitored germination daily from initial soaking until the germination peak was observed, after which monitoring was every other day. A seed was considered germinated and removed from the dish once the radicle protruded the testa. In flooded conditions, the number of sunk seeds and whether germinated seeds were sunk was recorded. After 55 d, germination

had reached a plateau, and we started the second phase of the experiment.

To initiate the recovery phase, ungerminated seeds previously exposed to 12.5, 25, or 45 dS/m were removed from salinity treatments and placed in freshwater at the same water level condition. The recovery phase lasted 21 d. We monitored germination daily until germination peak was observed, and then monitored every other day. Embryo viability of all ungerminated seeds was tested with a tetrazolium (2,3,5-triphenyl-2H-tetrazolium chloride) solution at 0.1% (Porter et al. 1947).

Statistical analyses

Predicted means and analysis of variance.—We performed all data analyses with R version 3.6.3 (R Core Team 2020). For both experimental phases, we compared the estimated final germination percentage (FGP) between population, salinity, and water level using a glm fitted with a quasibinomial distribution and logit link function. We estimated the time to reach 50% germination (T_{50}) by fitting a log-logistic model to cumulative data with package *drc* (Ritz et al. 2015). Differences among salinity, water level, and population were established by comparing the overlapping of 95% confidence intervals from the predicted values of the models.

Data homoscedasticity and normality of residuals were checked using Levene's and Shapiro's tests, respectively. Data were square-root-transformed if necessary to meet analysis of variance assumptions. Different ANOVAs were performed using the package *car* (Fox and Weisberg 2019). One-way ANOVAs were performed on average seed mass and seed coat thickness to test for population effect. A one-way ANOVA was also performed with salinity as grouping factor on a combination of the final germination percentages of seeds exposed to freshwater during the experiment and of the germination percentages obtained after the recovery for seeds that had been exposed to 12.5, 25, and 45 dS/m, to test whether seeds recovered at a similar level than whether they had been exposed to freshwater only. A three-way ANOVA with population, salinity level, water level, and their interactions was performed on embryo viability. A two-way ANOVA was performed on proportion of sank seeds as function of population and salinity.

Tukey's HSD test was applied using package *agricolae* (de Mendiburu 2013) for multiple comparisons when P values were significant (≤ 0.05). Using Student's t -tests, we also compared populations for the proportions of germinated sank and buoyant seeds, and proportion seeds that sunk in freshwater. All plots were generated using *ggplot2* (Wickham 2016).

Hydrotime model.—Water potential Ψ of experimental saline solutions was calculated as follows: $\Psi = \Psi_p + \Psi_s$, where pressure potential $\Psi_p = 0$ for a solution at atmospheric pressure. Solute (or osmotic) potential is given by $\Psi_s = -iCRT$ (van't Hoff 1884), where i corresponds to the ionization constant, that is, the number of ions produced when dissolved in water (two for NaCl), C is molar concentration (mole/L), R is a pressure constant (0.0831 liter bar/mole °K), and T is temperature (°K) calculated based on the average air temperature during the experiment ($21.4 \pm 5.1^\circ\text{C}$). Although we used Instant Ocean sea salts to make the saline solutions, the calculation of Ψ_s was based on NaCl, the dominant salt. We obtained the following water potential values: 0 dS/m = 0 MPa, 12.5 dS/m = -0.83 MPa, 25 dS/m = -1.65 MPa, and 45 dS/m = -2.89 MPa.

Salinity reduces water availability, which is reflected in the decrease in osmotic potential (Ψ_s). The relationship between water availability and germination rate (GR, rapidity of germination) is often described by a linear function (Bradford 1990, 2002): $\text{GR} = 1/t_g = (\Psi - \Psi_{b(g)})/\theta$ or $\theta = (\Psi - \Psi_{b(g)})t_g$, where t_g is time to germination percentile g , $\Psi_{b(g)}$ is the base water potential, that is, the Ψ threshold at or below which a seed ceases to germinate, and θ is the hydrotime constant (in MPa hours). Individual seeds vary in their $\Psi_{b(g)}$, and this variation among different percentiles within a seed population can be explored through population-based threshold models (Bradford 2002, Finch-Savage 2004). Rearranging the equation as $\Psi_{b(g)} = \Psi - (\theta/t_g)$, and assuming $\Psi_{b(g)}$ is normally distributed (but see Mesgaran et al. 2013), cumulative germination over time can be described by $g(\Psi, t_g) = \Phi(\Psi_{b(g)}, \Psi_{b(50)}, \Psi_\sigma)$, where Φ is the cumulative distribution function of a normal distribution, $\Psi_{b(50)}$ is the median base water potential, and σ is its standard deviation. For each population, the hydrotime model was fitted to the cumulative

germination data obtained at 0 and 12.5 dS/m for which sufficient germination existed to fit the model.

RESULTS

Vivipary

At BC, we observed precocious seed germination in capsules still attached to plants (vivipary), with germination of 0.18, 0.27, and 0.63% of the seeds collected in fall 2017, 2018, and 2019, respectively (Appendix S1: Fig. S4). No viviparous seeds were found in capsules collected from CS, and no vivipary was observed on plants in the field at CS, where soil and water salinity were 5–16 and 25–35 dS/m, respectively, during fall seed maturity and dispersal stage (Appendix S1: Fig. S1).

Seed morphological characteristics

Dry seed mass was 15% greater for BC than for CS (63 mg vs. 54 mg; Appendix S1: Table S1). Thickness of seed coats from CS was 6% greater than seed coats from BC (Appendix S1: Table S1, Figs. S5, S6).

Impact of experimental salinity and water levels on germination

Salinity drove differences in final germination percentages, independent of water level. Seeds exposed to freshwater presented the highest FGP (~96%, Fig. 1A). Those at 12.5 dS/m germinated up to 56%, a percentage 42% lower than that of seeds in freshwater (Fig. 1A). Very few seeds (1–3) germinated at 20 and 35 dS/m, and glm-estimated final germination percentages (5% and <0.1%, respectively) were not significantly different.

Seeds exposed to freshwater in flooded conditions germinated quickly, reaching 50% germination fraction in ~10 d, while seeds in moist freshwater treatments achieved this fraction within ~12 d (Fig. 1B). Seeds at 12.5 dS/m presented a T50 of 28 d in flooded conditions and 49 d in moist conditions. Seeds from BC exposed to freshwater and to 12.5 dS/m in flooded conditions reached 50% of germination 2.5 d sooner on average than those from CS (Fig. 1B).

Germination recovery from salinity exposure

Seeds formerly exposed to 12.5 dS/m increased their FGP an average of 16 percentage points

after exposure to freshwater (Fig. 1C). Nonetheless, seeds previously exposed to moist conditions at 12.5 dS/m reached a recovery FGP of ~64%, and ~80% FGP in flooded conditions. Seeds initially exposed to even higher salinity levels, 25 and 45 dS/m, also recovered with up to 78% germination on average, equivalent to seeds initially exposed to 12.5 dS/m in flooded conditions (Fig. 1C). However, these FGPs were still significantly lower than those of seeds exposed to 0 dS/m during the salinity experiment (one-way ANOVA, Appendix S1: Table S2).

Seeds formerly exposed to 25 dS/m germinated up to 50% faster than those initially exposed to 45 dS/m (2 d vs. 5 d; Fig. 1D). Seeds from BC initially exposed to 45 dS/m in flooded conditions had a T50 of 4.1 d, while T50 of those from CS was 5.6 d (Fig. 1D).

Hydrotime model

The model estimated hydrotime constants for seeds of *I. pseudacorus* were 10.82 MPa h for BC and 11.79 MPa h for CS. The normal distribution of base water potentials was similar between study populations (Fig. 2). Median base water potential was –1.16 MPa for BC and –1.12 MPa for CS. The water potential threshold at or above which 90% of seeds can germinate was –0.67 MPa (~10 dS/m) for BC and –0.59 MPa (~9 dS/m) for CS. For both populations, a water potential of –1.66 (~25 dS/m) predicts 10% of germinated seeds (Fig. 2, Appendix S1: Table S3).

Embryo viability

Salinity explained 15% of the variance in embryo viability (three-way ANOVA, Table 1). Seeds formerly exposed to 12.5 dS/m showed an embryo viability of ~84%, overall greater than that of those exposed to 0, 25, and 45 dS/m (~59%; Appendix S1: Fig. S7). There was an interaction between water level and population with seeds from CS presenting an embryo viability 1.6-fold lower than those from BC in moist conditions (Appendix S1: Fig. S7).

Seed buoyancy

Depending on source population, between 19% and 28% of seeds sunk when in flooded conditions in freshwater (Fig. 3), among which

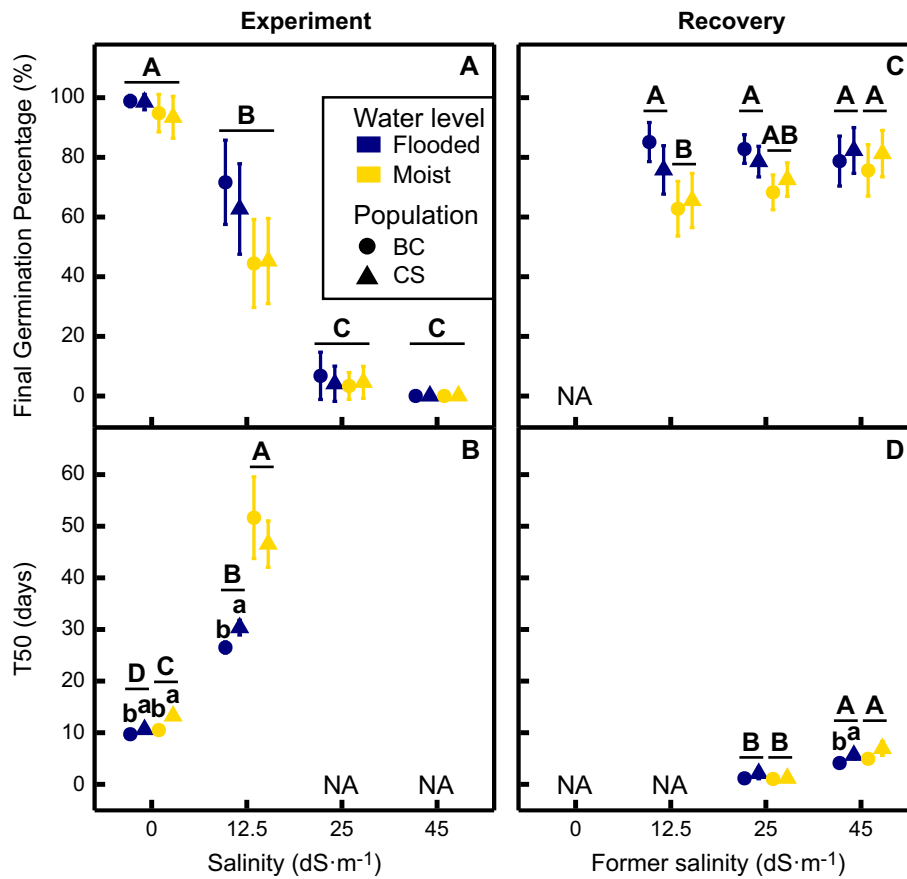


Fig. 1. Predicted means for final germination percentage and for T50 ($\pm 95\%$ CI) for seeds of two invasive populations of *Iris pseudacorus* exposed to four salinity levels and two water level conditions during a 55-d experiment (A, B) and exposed to freshwater during a 21-d recovery phase (C, D). Different capital letters indicate significant differences for salinity or for the interaction between salinity and water levels, and small letters indicate significant differences among populations. Error bars not visible are smaller than the points. NA is not applicable.

about 92% germinated (Appendix S1: Fig. S8), a proportion similar to that observed for floating seeds (95%; Student's *t*-test, Appendix S1: Table S4). For seeds exposed to 12.5 dS/m, ~13% sunk (15% for BC, 10% for CS), among which 60% germinated for BC and 86% for CS. In comparison, for buoyant seeds at 12.5 dS/m, 87% of BC seeds germinated and 67% of CS seeds germinated (Appendix S1: Table S4). When exposed to freshwater for recovery, the proportion of sunk seeds slightly increased for both populations, no sunken seeds germinated, and only 15% of buoyant seeds germinated (Fig. 3). At 25 and 45 dS/m salinity, ~10% of seeds sunk during the experiment for both populations, and none germinated. Upon transfer of seeds to freshwater, the

proportion of sunken seeds increased up to 21% and 27% for BC seeds initially exposed to 25 and 45 dS/m, respectively, and up to 13% and 18% for CS seeds initially exposed to 25 and 45 dS/m (Fig. 3). Among these sunken seeds, 55% germinated for BC and 89% for CS for those that had been exposed to 25 dS/m, and 32% germinated for BC, and 81% for CS when they had been exposed to 45 dS/m. For seeds that had been exposed to 25 and 45 dS/m, germination was lower for sunken seeds compared with floating seeds, among which ~88% germinated during freshwater recovery (Appendix S1: Fig. S8). When exposed to freshwater, seeds from CS were less buoyant than those from BC (Student's *t*-test: $P = 0.045$). However, the opposite was observed

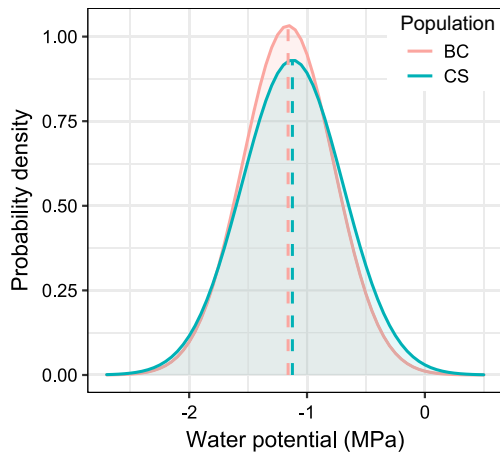


Fig. 2. Probability density of the normal distribution of base water potentials estimated by the hydro-time model for two invasive populations of *Iris pseudacorus*. At a given water potential, the fraction of seeds represented by the relative area under the curve to the right will not be able to complete germination. Dashed lines represent median base water potential.

Table 1. Results of the three-way ANOVA performed on the variable embryo viability for seeds of two invasive populations of *Iris pseudacorus* exposed to four salinity levels and two water level conditions (experiment) and then to freshwater (recovery).

Factors	df	Percentage of total sum of square	F	P
Population	1	4.1	4.39	0.04
Salinity	3	15.1	5.44	0.002
Water level	1	0.1	0.13	0.72
Population:salinity	3	2.9	1.05	0.38
Population:water level	1	9.4	10.14	0.002
Salinity:water level	3	0.1	0.05	0.98
Population:salinity:water level	3	5.1	1.85	0.15
Residuals	68	63.1		

Note: Significant results appear in boldface.

when seeds were recovering from exposure to salinity: Seeds from CS were significantly more buoyant than those from BC (Fig. 3; two-way ANOVA, Appendix S1: Table S5).

DISCUSSION

In this study, we examined the germination responses of *I. pseudacorus* to salinity and water level using seeds from two invasive intertidal populations from habitats with contrasted salinities. The salinity threshold impeding germination was between 9 and 25 dS/m for most seeds from both source populations. In the experiment, we observed almost no germination at 25 and 45 dS/m, but documented significant recovery germination after seed exposure to freshwater. There were distinct differences in germination velocity, buoyancy, and seed coat thickness between *I. pseudacorus* study populations. Our results demonstrate that after 55 d in seawater, buoyant seeds of *I. pseudacorus* retain their ability to germinate, and germinate quickly with freshwater exposure.

Potential for invasive colonization following seaborne dispersal

Seed germination fraction decreased as salinity level increased, in accordance with our first hypothesis. A salinity of 12.5 dS/m decreased germination by 30–50%, and exposure to 25 or 45 dS/m almost completely impeded the germination process. Although they did not recover thoroughly, there was a high degree of recovery for quiescent seeds formerly exposed to the highest salinity levels, in accordance with observations of *I. pseudacorus* by Sutherland (1990). Although rare, germination of seeds exposed to 25 or 45 dS/m occurred during our experiment. Such germination events could establish a new population, provided the seedling survives and establishes under such stressful conditions. Rare events such as this may further contribute to species spread with potential for populations acclimated to highly saline environments. Here, the application of high salt stress conditions for 55 d did not significantly affect embryo dormancy or viability, while other aquatic plant species, including some halophytes, have low capacity for post-salinity exposure recovery (Greenwood and MacFarlane 2006, Muñoz-Rodríguez et al. 2017).

Seed dispersal represents a fundamental process in invasion ecology and plant community assembly. Long-distance dispersal events contribute to the spread of plant populations and their colonization of unoccupied habitats

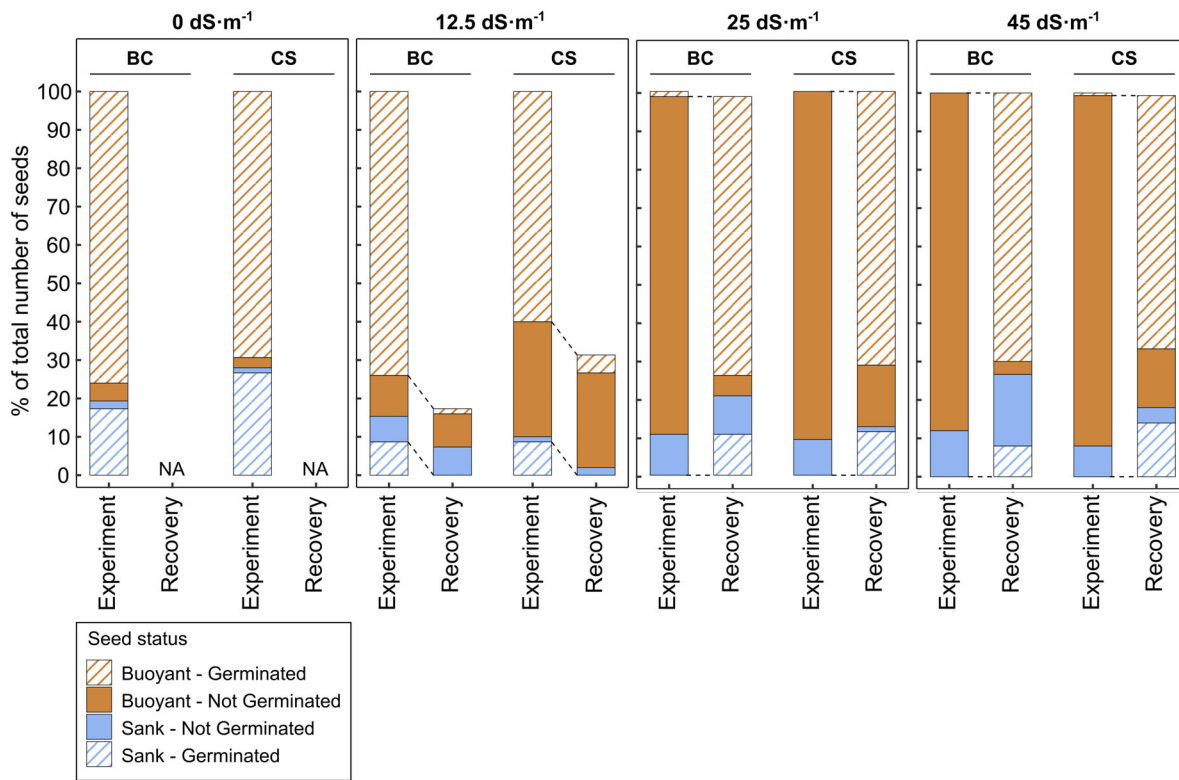


Fig. 3. Proportion of seeds of *Iris pseudacorus* that sank or were buoyant and their germination status when they were exposed to four different salinity levels (experiment), followed by a freshwater recovery period in flooded conditions, for two populations: San Joaquin River at Buckley Cove (BC) and Carquinez Strait at Glen Cove (CS). NA is not applicable.

(Nathan et al. 2008), and distributions of many plant species can be explained by events of long-distance dispersal by ocean currents (Jordan 2001). Long-lasting buoyancy and hydrochorous dispersal of *I. pseudacorus* seeds via tides suggest they are likely candidates for long-distance transport by ocean currents. These factors, combined with high embryo survival to seawater exposure, could support the expansion of the invasive range of the species through the establishment of new populations disjunct from source populations (Fig. 4). Dispersal of vegetative and sexual propagules via hydrochory is common for many aquatic and riverine plant species (Boedeltje et al. 2003), and hydrologic connectivity increases the vulnerability of watersheds to biological invasions (Pyšek and Prach 1993). When dispersed propagules reach the sea, ocean currents can transport buoyant seeds over

hundreds of kilometers (Nathan et al. 2008). Therefore, concerns regarding the potential abilities of *I. pseudacorus* to further disperse in the San Francisco Estuary and beyond are strengthened by the present study. SLR will modify tidal currents (Khojasteh et al. 2020), and the increase in high-tide flooding could provide new opportunities for the species to spread and reach new locations.

Salinity threshold and field observations

According to the hydrotime model, the sensitivity threshold to salinity was comparable between study populations. Most seeds of invasive *I. pseudacorus* were able to germinate between 9 and 25 dS/m, consistent with low experimental germination response we recorded in >25 dS/m salinity. The decrease in germination from 12.5 dS/m in our experiment was in

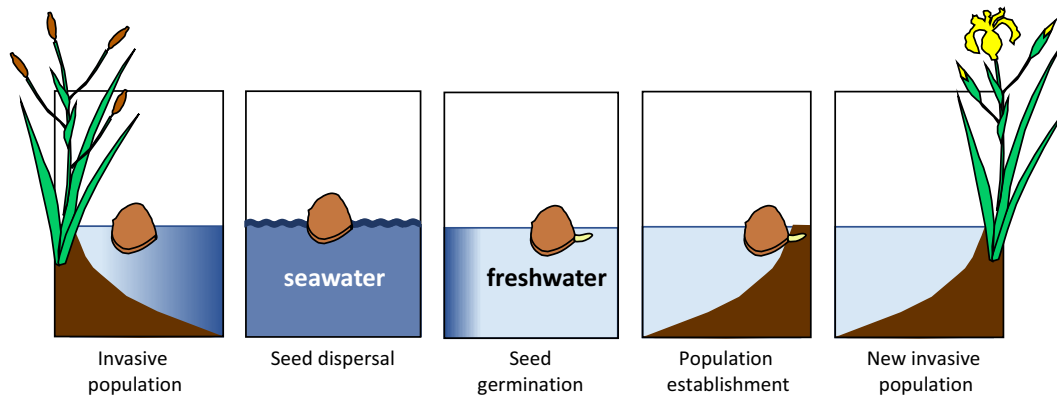


Fig. 4. Seeds from invasive populations of *Iris pseudacorus* can germinate after exposure to seawater. Seaborne seeds may allow the establishment of new populations distant from source population.

accordance with incidental observations of seedling emergence recorded at field sites. For example, at BC we observed 40–50 newly emerged seedlings in early spring of 2018–2019, while at CS where salinity was higher, only four seedlings were observed and did not survive (B. J. Grewell, *personal observation*). This is also consistent with an experiment performed on horticultural cutting of *I. pseudacorus* showing a decrease in seedling survival from above 12.5 dS/m (Zhao et al. 2015). In addition, salt stress resulted in a two-fold reduction in biomass production by invasive *I. pseudacorus* over an 18-month period when rhizomes were exposed to 10 dS/m, compared with freshwater (Mopper et al. 2016). Embryo viability of seeds formerly exposed to 12.5 dS/m was greater than that of those from 0, 25, and 45 dS/m. Therefore, for some seeds, exposure to 12.5 dS/m may have induced a secondary physiological dormancy not broken by exposure to freshwater. We demonstrated that some fractions of seed populations tolerate higher salinity levels than others and documented germination of a few seeds at 25 and 45 dS/m. This suggests *I. pseudacorus* presents capacities to germinate during summer and fall, when salinity is higher at our study sites (Appendix S1: Fig. S1); yet, germination at this time would expose seedlings to high osmotic stress levels in sediments. However, *I. pseudacorus* germination was accelerated and synchronized among seeds at freshwater after salinity exposure, comparable to many halophytes (Muñoz-Rodríguez et al. 2017). This response is key in a glycophyte species such as *I. pseudacorus* to germinate during windows of

germination at low salinities (Woodell 1985). Our hydrotime model provides a first estimate of seed population tolerance to salinity for this species. Such population-based models represent powerful tools to predict timing of seedling emergence depending on various environmental conditions, and are hence increasingly used for ecological and management purposes for crop and invasive species (Batlla and Benech-Arnold 2005, Boddy et al. 2012).

We observed a small fraction of germinated seeds of *I. pseudacorus* within ripe fruits on mother plants at BC, at the end of the summer and at fall. Vivipary is considered an adaptation to changing saline conditions (Cota-Sanchez et al. 2007). However, here vivipary was not observed for *I. pseudacorus* at CS, where plants are exposed to greater salinity. Germinated seeds were also observed floating on the water surface during the fall season at BC where benign freshwater conditions persist throughout the year. Fall germination of this species has previously been mentioned (Guppy 1912, Nakashima and Oki 2005). Therefore, it appears that a fraction of the seed population of *I. pseudacorus* may not present primary dormancy.

New insights about water level effects and seed buoyancy of I. pseudacorus

Counter to our second hypothesis, seeds did not germinate better in moist conditions than in flooded conditions. Instead, seeds in moist conditions had a lower germination capacity at 12.5 dS/m and germinated slowly both at 0 and 12.5 dS/m, which could result from a lower and

slower water imbibition, perhaps due to low seed coat–water contact. Indeed, seeds of *I. pseudacorus* are relatively large and need to imbibe ample water before they germinate. We controlled for this factor by soaking all seeds for one week at the beginning of the experiment. Although water level had no effect on embryo viability of ungerminated seeds, seeds of *I. pseudacorus* may partially dehydrate when not in full-flooded conditions. Seedlings from seeds that germinate at the soil surface may desiccate and die prematurely (Kleemann et al. 2007). Seed burial at shallow soil depth can prevent desiccation of seeds (Baskin and Baskin 2014) and emergent seedlings.

Our results differ from those of Coops and Vanderveelde (1995), who found seeds of *I. pseudacorus* germinated uniquely when placed on a humidified soil surface, and not on saturated soil or when under 5 cm of water. In addition, they observed no germination of *I. pseudacorus* when seeds were free-floating in similar conditions to our flooded treatment. Furthermore, they did not notice any sunk seeds over a 42-d period. These differences with our results may be related to local adaptation, or other unknown genetic differentiation among global populations. In agreement with our results, Sutherland (1990) saw that at least 95% of seeds floated for two months, and van den Broek et al. (2005) reported a gradual decrease in the numbers of floating seeds over time.

Signs of potential acclimation to saline environment

We demonstrated interpopulation differences among seeds during both the salinity exposure and recovery phases of our experiment. Seeds of *I. pseudacorus* from CS, exposed to higher salinities in the field, germinated slower than those from BC. This could be explained by the thicker testa measured on seeds from CS, a potentially advantageous trait to maintain seed survival when environmental conditions are conducive to seed dehydration. In contrast to our results, seeds from populations of *I. hexagona*, native to North American wetlands, germinated earlier and in greater numbers when mother plants were growing in low-brackish water rather than freshwater (Van Zandt and Mopper 2004).

Similar to measured weights of seeds from BC and CS, plants of facultative halophytes produce

lighter seeds in maternal environments with high salinity than those produced in nonsaline habitat (El-Keblawy et al. 2017). Overall, lower seed mass is commonly observed when maternal plants are exposed to salinity (Crean and Marshall 2009).

Finally, there were differences in seed buoyancy depending on population and salinity conditions, which may be related to changes in the seed density and in the air space between the endosperm and the seed coat (Romero-Méndez et al. 2018). Therefore, our results revealed physical differences in seeds of *I. pseudacorus* with a decrease in mass and an increase in the testa thickness that may change germination and buoyancy properties. These differences may be due to maternal effects or to local adaptation in response to the different salinity conditions at population site, but they could also correspond to genetic variations (e.g., genetic drift) between populations. Although our study tested seeds of populations present at extreme ends of an estuary gradient, these two populations alone are not sufficient to describe interpopulation variability, and they may not be representative of the most extreme responses and physical modifications that can exist for seeds of *I. pseudacorus* in response to salinity. Additional investigations with a larger set of populations and controlling for maternal effects would be necessary to comprehend the potential of acclimation and adaptation of the species to salinity.

CONCLUSIONS

Improved understanding of dormancy-breaking and seed germination thresholds, crucial for the reintroduction of endangered species (Ribeiro et al. 2016), can be equally important for applied management to reduce the ecological impacts of invasive species threatening native taxa. Our results highlight the risk of establishment of *I. pseudacorus* populations via hydrochorous dispersal of buoyant seeds, including potential long-distance dispersal via seawater, and their potential germination in freshwater or saline wetland habitats distant from source populations in the invasive range (Fig. 4). Therefore, a management priority should be to remove plants and/or seed capsules prior to reproductive maturity to prevent the development of mature seed

and limit dispersal. Salinity increase expected from SLR could limit *I. pseudacorus* germination and therefore seedling establishment in habitats with aqueous salinity >25 dS/m. However, it is less likely that SLR and salinity increase could reduce the germination capacities of the species in habitats with salinity <25 dS/m. Therefore, increased salinity intrusion with SLR will reduce recruitment of *I. pseudacorus* and may facilitate invasive plant management efforts to achieve desired plant community composition. However, the community-level outcome will also depend on tolerances of established perennial stands and of interacting native and other invasive glyco-phytes to increasing salinity and tidal ranges (Hellmann et al. 2008). Our results confirm concerns regarding the ability of *I. pseudacorus* to spread in sensitive coastal wetland habitats, and provide important details to improve risk assessments and management to prevent further spread of *I. pseudacorus* in vulnerable tidal wetlands.

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