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°58ʹ38.1ʺ N, 121°22ʹ15.0ʺ 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 glycyphyte 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× 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 × 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 × two populations × four salinity levels × two water levels) were randomly distributed on benches in greenhouse conditions (supplemental lighting 280 μmol·m⁻²·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 con-
dition. The recovery phase lasted 21 d. We moni-
tored 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 germi-
nation percentage (FGP) between population,
salinity, and water level using a glm fitted with a
quasibinomial distribution and logit link func-
tion. We estimated the time to reach 50% germi-
nation (T50) 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 resid-
uals were checked using Levene’s and Shapiro’s
tests, respectively. Data were square-root-shap-
transformed if necessary to meet analysis of variance
assumptions. Different ANOVAs were per-
formed 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 per-
centages of seeds exposed to freshwater during
the experiment and of the germination percent-
ages 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 freshwa-
ter 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 com-
parisons when P values were significant (≤0.05).
Using Student’s t-tests, we also compared popu-
lations for the proportions of germinated sank
and buoyant seeds, and proportion seeds that
sunk in freshwater. All plots were generated
using ggplot2 (Wickham 2016).

**Hydrot ime model.**—Water potential \( \Psi \) of experi-
mental saline solutions was calculated as fol-
nows: \( \Psi = \Psi_p + \Psi_s \), where pressure potential
\( \Psi_p = 0 \) for a solution at atmospheric pressure.
Solute (or osmotic) potential is given by \( \Psi_s = -iT \Psi \) (van’t Hoff 1884), where \( i \) corre-
sponds 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) calcu-
lated based on the average air temperature dur-
ing the experiment (21.4 ± 5.1°C). Although we
used Instant Ocean sea salts to make the saline
solutions, the calculation of \( \Psi_s \) was based on
NaCl, the dominant salt. We obtained the fol-
lowing 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
(\( \Psi_s \)). The relationship between water availability
and germination rate (GR, rapidity of germina-
tion) is often described by a linear function
(Bradford 1990, 2002): \( GR = 1/t_g = (\Psi - \Psi_{b(g)})/\theta \)
or \( \theta = (\Psi - \Psi_{b(g)})t_g \), where \( t_g \) is time to germina-
tion percentile \( g \). \( \Psi_{b(g)} \) is the base water potential,
that is, the \( \Psi \) threshold at or below which a seed
cesses to germinate, and \( \theta \) is the hydrot ime
constant (in MPa hours). Individual seeds vary in
their \( \Psi_{b(g)} \) and this variation among different per-
centiles within a seed population can be explo-
red 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 germina-
tion over time can be described by \( g(\Psi, t_g) = \Phi \)
\( (\Psi_{b(g)}, \Psi_{b(50)}, \Psi_{s}), \) where \( \Phi \) is the cumulative
distribution function of a normal distribution, \( \Psi_{b(50)} \)
is the median base water potential, and \( \sigma \) is its
standard deviation. For each population, the
hydrot ime 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 (2% 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 FGP’s 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
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 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% germinated for CS seeds initially exposed to 25 and 45 dS/m. 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.
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).

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.

Fig. 2. Probability density of the normal distribution of base water potentials estimated by the hydrotime 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.

**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-Rodriguez 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.
(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

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**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.
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-Rodriguez et al. 2017). This response is key in a glycyphyte 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 Vandervelde (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-Mendez 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 glycophytes 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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**Literature Cited**

Baskin, C. C., and J. Baskin. 2014. Seeds. Ecology, biogeography, and evolution of dormancy and germination. Second edition. Academic Press, San Diego, California, USA. https://doi.org/10.1016/C2013-0-00597-X

Batlla, D., and R. L. Benech-Arnold. 2005. Changes in the light sensitivity of buried *Polygonum aviculare* seeds in relation to cold-induced dormancy loss: development of a predictive model. New Phytologist 165:445–452.

Boddy, L. G., K. J. Bradford, and A. J. Fischer. 2012. Population-based threshold models describe weed germination and emergence patterns across varying temperature, moisture and oxygen conditions. Journal of Applied Ecology 49:1225–1236.

Boedeltje, G., J. P. Bakker, R. M. Bekker, J. M. Van Groenendael, and M. Soesbergen. 2003. Plant dispersal in a lowland stream in relation to occurrence and three specific life-history traits of the species in the species pool. Journal of Ecology 91:855–866.

Bradford, K. J. 1990. A water relations analysis of seed germination rates. Plant Physiology 94:840–849.

Bradford, K. J. 2002. Applications of hydrothermal time to quantifying and modeling seed germination and dormancy. Weed Research 50:248–260.

Brown, L., W. Kimmerer, J. L. Conrad, S. Lesmeister, and A. Mueller-Solger. 2016. Food Webs of the Delta, Suisun Bay, and Suisun Marsh: an update on current understanding and possibilities for management. San Francisco Estuary and Watershed Science 14:1–41.

Chambers, J. C., and J. A. MacMahon. 1994. A day in the life of a seed: movements and fates of seeds and their implications for natural and managed systems. Annual Review of Ecology and Systematics 25:263–292.

Colmer, T. D., and L. A. C. J. Voosenek. 2009. Flooding tolerance: suites of plant traits in variable environments. Functional Plant Biology 36:665.

Contreras-Cruzado, I., M. D. Infante-Izquierdo, B. Marquez-Garcia, V. Hermoso-Lopez, A. Polo, F. J. J. Nieva, J. Bautista Cartes-Barroso, J. M. Castillo, and A. Munoz-Rodriguez. 2017. Relationships between spatio-temporal changes in the sedimentary environment and halophytes zonation in salt marshes. Geoderma 305:173–187.

Coops, H., and G. Vandervelde. 1995. Seed dispersal, germination and seedling growth of 6 helophyte species in relation to water-level zonation. Freshwater Biology 34:13–20.

Cota-Sanchez, J. H., A. Reyes-Olivas, and B. Sanchez-Soto. 2007. Vivipary in coastal Cacti: a potential reproductive strategy in halophytic environments. American Journal of Botany 94:1577–1581.

Crean, A. J., and D. J. Marshall. 2009. Coping with environmental uncertainty: dynamic bet hedging as a maternal effect. Philosophical Transactions of the Royal Society B: Biological Sciences 364:1087–1096.

Dalziel, E. L., W. Lewandrowski, and D. J. Merritt. 2020. Increased salinity reduces seed germination and impacts upon seedling development in *Nymphae a L.* (*Nymphaeaceae*) from northern Australia’s freshwater wetlands. Aquatic Botany 165:103235.
de Mendiburu, F. 2013. agricolae: statistical Procedures for Agricultural Research. R package version 1.2-1. https://cran.r-project.org/web/packages/agricolae/index.html

Donohue, K., R. Rubio de Casas, L. Burghardt, K. Kovach, and C. G. Willis. 2010. Germination, post-germination adaptation, and species ecological ranges. Annual Review of Ecology, Evolution, and Systematics 41:293–319.

El-Keblawy, A., S. Gairola, A. Bhatt, and T. Mahmoud. 2017. Effects of maternal salinity on salt tolerance during germination of *Suaeda aegyptiaca*, a facultative halophyte in the Arab Gulf desert. Plant Species Biology 32:45–53.

Eller, F., et al. 2017. Cosmopolitan species as models for ecophysiological responses to global change: The common reed *Phragmites australis*. Frontiers in Plant Science 8:1833.

Engels, J. G., and K. Jensen. 2010. Role of biotic interactions and physical factors in determining the distribution of marsh species along an estuarine salinity gradient. Oikos 119:679–685.

Fenner, M. K., and K. Thompson. 2005. The ecology of seeds. Second edition. Cambridge University Press, Cambridge, UK. https://doi.org/10.1017/CBO9780511614101

Finch-Savage, W. E. 2004. The use of population-based threshold models to describe and predict the effects of seedbed environment on germination and seedling emergence of crops. Pages 51–96 in Handobook of seed physiology: applications to agriculture. Haworth Press, New York, New York, USA.

Fox, J., and S. Weisberg. 2019. An R companion to applied regression. Third edition. Sage, Thousand Oaks, California, USA.

Gallego-Trév, B., B. J. Grewell, C. J. Futrell, R. E. Drenovsky, and J. M. Castilo. 2019. Interactive effects of salinity and inundation on native *Sparrtina foliosa*, invasive *S. densiflora*, and their hybrid from San Francisco Estuary, California. Annals of Botany 125:377–389.

Gaskin, J. F., M. L. Pokorny, and J. M. Mangold. 2016. An unusual case of seed dispersal in an invasive aquatic; yellow flag iris (*Iris pseudacorus*). Biological Invasions 18:2067–2075.

Gervazoni, P., A. Sosa, C. Franceschini, J. Coetzee, A. Falthhauser, D. Fuentes-Rodriguez, A. Martinez, and M. Hill. 2020. The alien invasive yellow flag (*Iris pseudacorus* L.) in Argentinian wetlands: assessing geographical distribution through different data sources. Biological Invasions 22:3183–3193.

Goodman, A. M., G. G. Ganf, H. R. Maier, and G. C. Dandy. 2011. The effect of inundation and salinity on the germination of seed banks from wetlands in South Australia. Aquatic Botany 94:102–106.

Greenway, H., and R. Munns. 1980. Mechanisms of salt tolerance in nonhalophytes. Annual Review of Plant Physiology 31:149–190.

Greenwood, M. E., and G. R. MacFarlane. 2006. Effects of salinity and temperature on the germination of *Phragmites australis*, *Juncus kraussii*, and *Juncus acutus*: implications for estuarine restoration initiatives. Wetlands 26:854–861.

Guppy, H. B. 1912. Studies in seeds and fruits. Williams and Norgate, London, UK.

Hayasaka, D., S. Fujiwara, and T. Uchida. 2018. Impacts of invasive *Iris pseudacorus* L. (yellow flag) establishing in an abandoned urban pond on native semi-wetland vegetation. Journal of Integrative Agriculture 17:1881–1887.

Hellmann, J. J., J. E. Byers, B. G. Bierwagen, and J. S. Dukes. 2008. Five potential consequences of climate change for invasive species. Conservation Biology 22:534–543.

Infante-Izquierdo, M. D., J. M. Castilo, B. J. Grewell, F. J. F. Nieva, and A. F. Muñoz-Rodríguez. 2019. Differential effects of increasing salinity on germination and seedling growth of native and exotic invasive cordgrasses. Plants 8:372.

Janousek, C. N., and C. L. Folger. 2013. Inter-specific variation in salinity effects on germination in Pacific Northwest tidal wetland plants. Aquatic Botany 111:104–111.

Jordan, G. J. 2001. An investigation of long-distance dispersal based on species native to both Tasmania and New Zealand. Australian Journal of Botany 49:333.

Khojasteh, D., S. Hottinger, S. Felder, G. De Cesare, V. Heimhuber, D. J. Hanslow, and W. Glamore. 2020. Estuarine tidal response to sea level rise: the significance of entrance restriction. Estuarine, Coastal and Shelf Science 244:106941.

Kleemann, S. G. L., B. S. Chauhan, and G. S. Gill. 2007. Factors affecting seed germination of perennial wall rocket (*Diptlotaxus tenuifolius*) in southern Australia. Weed Science 55:481–485.

Mesgaran, M. B., H. R. Marshhabi, H. Alizadeh, J. Hunt, K. R. Young, and R. D. Cousens. 2013. Importance of distribution function selection for hydrothermal time models of seed germination. Weed Research 53:89–101.

Mopper, S., K. C. Wiens, and G. A. Goranova. 2016. Competition, salinity, and clonal growth in native and introduced irises. American Journal of Botany 103:1575–1581.

Muñoz-Rodríguez, A. F., I. Sanjós, B. Márquez-García, M. D. Infante-Izquierdo, A. Polo-Avila, F. J. J. Nieva, and J. M. Castilo. 2017. Germination
syndromes in response to salinity of Chenopodiaceae halophytes along the intertidal gradient. Aquatic Botany 139:48–56.
Nakashima, Y., and Y. Oki. 2005. Relationship between the position effects, the seed shapes, the dehiscent grades of the capsules and the seed coat crack and the autumn emergence of Iris pseudacorus seed. Journal of Weed Science and Technology 50:276–284.
Nathan, R., F. M. Schurr, O. Spiegel, O. Steinitz, A. Trakhtenbrot, and A. Tsoar. 2008. Mechanisms of long-distance seed dispersal. Trends in Ecology and Evolution 23:638–647.
Nielsen, D. L., M. A. Brock, M. Vogel, and R. Petrie. 2008. From fresh to saline: a comparison of zooplankton and plant communities developing under a gradient of salinity with communities developing under constant salinity levels. Marine and Freshwater Research 59:549.
Parihar, P., S. Singh, R. Singh, V. P. Singh, and S. M. Prasad. 2015. Effect of salinity stress on plants and its tolerance strategies: a review. Environmental Science and Pollution Research 22:4056–4075.
Parker, V. T., and K. E. Boyer. 2019. Sea-level rise and climate change impacts on an urbanized pacific coast estuary. Wetlands 39:1219–1232.
Pathikonda, S., A. S. Ackleh, K. H. Hasenstein, and S. Mopper. 2009. Invasion, disturbance, and competition: modeling the fate of coastal plant populations. Conservation Biology 23:164–173.
Porter, R. H., M. Durrell, and H. J. Romm. 1947. The use of 2,3,5-triphenyl-tetrazoliumchloride as a measure of seed germinability. Plant Physiology 22:149–159.
Pyšek, P., and K. Prach. 1993. Plant invasions and the role of riparian habitats: a comparison of four species alien to central Europe. Pages 254–263 in Ecosystem management. Springer, New York, New York, USA.
R Core Team. 2020. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Austria, Vienna. https://www.R-project.org/
Ribeiro, G. V. T., A. L. Teixido, N. P. U. Barbosa, and F. A. O. Silveira. 2016. Assessing bias and knowledge gaps on seed ecology research: implications for conservation agenda and policy. Ecological Applications 26:2033–2043.
Ritz, C., F. Baty, J. C. Streibig, and D. Gerhard. 2015. Dose-response analysis using R. PLOS ONE 10: e0146021.
Romero-Méndez, U., J. L. Becerra-López, C. García-De la Peña, J. J. Martínez-Ríos, A. Czaja, G. Muro, and J. Sánchez. 2018. Hydrochory in Astrophytum coahuilense: experiment to identify structures that provide its buoyancy. Polibotánica 46:149–157.
Sutherland, W. J. 1990. Iris pseudacorus L. Journal of Ecology 78:833–848.
Sutherland, W. J., and D. Walton. 1990. The changes in morphology and demography of Iris pseudacorus L. at different heights on a saltmarsh. Functional Ecology 4:655–659.
van den Broek, T., R. van Diggelen, and R. Bobbink. 2005. Variation in seed buoyancy of species in wetland ecosystems with different flooding dynamics. Journal of Vegetation Science 16:579–586.
Van Zandt, P. A., and S. Mopper. 2004. The effects of maternal salinity and seed environment on germination and growth in Iris hexagona. Evolutionary Ecology Research 6:813–832.
Van Zandt, P. A., M. A. Tobler, E. Mouton, K. H. Hasenstein, and S. Mopper. 2003. Positive and negative consequences of salinity stress for the growth and reproduction of the clonal plant, Iris hexagona. Journal of Ecology 91:837–846.
Wickham, H. 2016. ggplot2: elegant graphics for data analysis. Springer, New York, New York, USA. https://doi.org/10.1002/recl.1884031003
Walck, J. L., S. N. Hidayati, K. W. Dixon, K. Thompson, and P. Poschlod. 2011. Climate change and plant regeneration from seed. Global Change Biology 17:2145–2161.
Woodell, S. R. J. 1985. Salinity and seed germination patterns in coastal plants. Pages 223–229 in Ecology of coastal vegetation. Springer, Dordrecht, The Netherlands.
Zhao, H., F. Wang, and M. Ji. 2015. Brackish Eutrophic Water Treatment by Iris pseudacorus L.-Planted Microcosms: physiological Responses of Iris pseudacorus L. to Salinity. International Journal of Phytoremediation 17:814–821.
Zhao, Z., L. Yuan, W. Li, B. Tian, and L. Zhang. 2020. Re-invasion of Spartina alterniflora in restored saltmarshes: seed arrival, retention, germination, and establishment. Journal of Environmental Management 266:110631.

**Supporting Information**

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