

Variability in seed salinity tolerance in an island coastal community

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- **Background and Aims** Islands, with their long coastlines and increased vulnerability to sea level rise, offer compelling opportunities to investigate the salinity tolerance of coastal plants. Seeds are generally more vulnerable than other plant stages to increased stressors. The aim of this study was to characterize salinity tolerance during germination across a diverse pool of 21 species from 14 plant families found in coastal communities throughout the Hawaiian Islands in order to increase our general understanding of coastal plant ecology for conservation and restoration.
- **Methods** Seeds of each species were exposed to unfiltered/untreated seawater (35 ppt total salinity) and two salinity treatments (10 and 20 ppt) in which the seawater was diluted with distilled water, and germination percent and timing were compared to seeds in a distilled water control. Non-germinated seeds were then tested for recovery germination. We quantified and compared germination percent, time and recovery among species and across salinity levels and tested for heterogeneity related to seed size, dormancy class, habit and threatened status.
- **Key Results** Although salinity tolerance varied considerably among species, salinity exposure generally reduced and delayed germination. The greatest effects were detected at higher salinity levels. Recovery germination overall was higher for seeds that had been exposed to higher salinity. None of the factors we explored emerged as predictors of salinity tolerance except seed mass, which tended to enhance germination at higher salinity.
- **Conclusions** Species responses to salinity exposure indicate high vulnerability of coastal systems to increased salinity stress, and variability among species could lead to shifts in community assembly and composition under sea level rise. These results can help guide coastal ecosystem conservation and restoration management decisions in the face of climate change.

Key words: Climate change, coastal community, coastal ecosystem, Hawai‘i, Hawaiian coastal plants, islands, salt tolerance, seed salinity tolerance.

INTRODUCTION

Coastal ecosystems are ecologically diverse, supporting heterogeneous habitat types that are home to a diversity of native species that are often endemic to these narrow habitats (Burke *et al.*, 2001; UNEP, 2006). Coastal ecosystems also provide regulating services that humans rely on, such as shoreline stabilization, protection from severe storms and nutrient cycling (UNEP, 2006; Martínez *et al.*, 2007). Moreover, coastal ecosystems provide cultural, recreational and provisional services to people worldwide.

Climate change is one of the dominant global threats to coastal ecosystems, with all low-lying coastal habitats at risk from current and ongoing sea level rise (UNEP, 2006). In a span of just over 100 years (from 1901 to 2018), global mean sea level rose by 0.20 m, and the rate of sea level rise has steadily increased and is projected to continue along this trajectory into the future (IPCC, 2021). Sea level rise poses various threats to coastal ecosystems and biological communities, including habit loss from increased coastal erosion as well as elevated

salinity stress due to coastal flooding and increased storm surges (Hanley *et al.*, 2020a; IPCC, 2021). Increased aridity due to reduced precipitation and elevated temperatures, or both, is expected to exacerbate the effects of sea level rise, leading to greater accumulation of salt ions in coastal habitats (Dasgupta *et al.*, 2015). Where suitable habitat exists inland, coastal plants may escape from increased salinization as sea level rise progresses by migrating inland via dispersal. However, this process is slow, and whether plants can withstand elevated salinity while migration occurs depends on salinity tolerance across all life stages. Salinity tolerance during seed germination and seedling establishment are particularly important as recruitment bottlenecks may reduce population stability (Harper, 1977; Del Vecchio *et al.*, 2020).

It is generally predicted that coastal plants have evolved salinity tolerance, enabling them to inhabit coastal environments despite constant exposure to salt spray and periodic exposure to high tides and storm events. Salinity stress interferes with plant performance indirectly via osmotic stress and directly via ion

imbalances and cellular ion toxicity. Salt-tolerant plants may express various mechanisms to overcome these distinct challenges to plant performance, although many of these pertain to vegetative plant stages (Munns and Tester, 2008; Zunzunegui *et al.*, 2017).

Seeds may also experience salinity stress via osmotic stress and direct toxicity, and hormonal disruption may also play a role as salinity induces abscisic acid, which can inhibit germination (Zhu, 2000, 2001). Osmotic stress during germination typically reduces the rate of water imbibition, leading to delayed germination under high salinity, but total germination can eventually reach high levels, particularly if salinity is reduced by exposure to freshwater (Heard and Ancheta, 2011; Guja *et al.*, 2013; El-Katony *et al.*, 2015). Delayed seed germination until seeds are exposed to fresh water conditions may be adaptive, ensuring seedlings have a greater chance of establishment during less harsh, lower salinity conditions (Llanes *et al.*, 2015; Raddi *et al.*, 2019; Malik *et al.*, 2022). In contrast to osmotic effects, salt toxicity resulting from the accumulation of sodium and chloride ions within the embryo may directly reduce plant function via disruption of ionic balance of potassium and calcium, and interference with protein degradation from stored reserves during germination (Debez *et al.*, 2012; Guja *et al.*, 2013). Ion toxicity is thus more likely to be fatal to seeds compared with osmotic stress, resulting in a failure of recovery germination upon exposure to freshwater (Ignaciuk and Lee, 1980; Saeed *et al.*, 2011).

Assessing salinity effects on germination has been a major focus of coastal plant research, with representative species sampled from many coastal regions around the world. However, in most studies, only one or a few species are examined, and so the consequences of increased salinity on community assembly and composition remain difficult to predict. More comprehensive sampling of a greater number of co-occurring coastal plant species is needed for robust assessments of the vulnerability of coastal plant communities to sea level rise, and would reveal whether co-occurring species converge on similar levels of salinity tolerance, as might be predicted given their shared environmental conditions. Although uncommon, comparative studies testing salinity tolerance during germination have revealed considerable variability among coastal plant species (Woodell, 1985; Martínez *et al.*, 1992; Hanslin and Eggen, 2005; Guja *et al.*, 2010). In some cases, species variability in salinity tolerance leads to spatial segregation along salinity gradients correlated with distance from the sea (Martínez *et al.*, 1992; Del Vecchio *et al.*, 2020), while in other cases, variability occurs among plant species growing in close proximity (Woodell, 1985; Martínez *et al.*, 1992; Hanslin and Eggen, 2005), suggesting that despite shared abiotic environments, species can differ in seed salinity tolerance, probably as a result of intrinsic differences in physiology and life history.

Islands offer compelling opportunities to investigate salinity tolerance of coastal plants given their long coastlines and vulnerability to sea level rise. Despite their small area, islands are home to a disproportionate share of global biodiversity, and they suffer comparatively high rates of endangerment and extinction (Fernández-Palacios *et al.*, 2021). Sea level rise and associated increases in salinity are key threats to island coastal floras, yet relatively few studies have tested seed salinity tolerance in

island plants (Santo *et al.*, 2014, 2017; Del Vecchio *et al.*, 2018; Lum and Barton, 2020).

In the Hawaiian Islands, projections of sea level rise (Kopp *et al.*, 2014) and increased drought (Elison Timm *et al.*, 2015) will increase the level of salinity to which coastal plant species are exposed. By the year 2100, the projected probable range of sea level rise in the islands (based on projections for Honolulu, Hawai'i) is between 0.6 and 1.1 m, which is higher than projections for global mean sea level rise (Kopp *et al.*, 2014). When high wave events and coastal erosion are factored into models, the impacts of rising sea levels on Hawai'i's coastal lowlands from flooding are even more substantial (Anderson *et al.*, 2018). Furthermore, even though region-specific frequency and intensity of storms are difficult to model (Seneviratne *et al.*, 2012), the impact of storms in the islands will nonetheless be much greater with higher sea levels (Marra *et al.*, 2012).

Although some species may be able to tolerate these changes, the salinity tolerances of most Hawaiian coastal plant species during germination are unknown. Previous research has focused largely on vegetative stages, generally detecting weak salinity tolerance for native coastal species, particularly at the seedling stage (Alpha *et al.*, 1996; Goldstein *et al.*, 1996; Suarez, 2011; Lum and Barton, 2020). Moreover, previous studies have tested only a few species, precluding predictions for how sea level rise will affect coastal plant community assembly and composition. With the goal of characterizing coastal communities more holistically, we tested salinity tolerance during germination in 21 species from 14 plant families (Table 1). Characterizing salinity tolerance during germination across this diverse species pool will offer insights into community-scale vulnerability to sea level rise in a diverse island flora, contributing to our understanding of coastal plant ecology that is critical for the conservation of these unique ecosystems.

MATERIALS AND METHODS

Species selection and seed preparation

Species were selected with the goal of representing the diversity of native plants that occur together in Hawai'i's coastal habitats (Table 1). They include common and widespread species, found in dunes and other coastal habitat such as cliffs and scrub, as well as rare species. For insights into possible effects of salinity on invasion, we included one of the most pervasive invasive species in Hawai'i's coastal habitats, *Leucaena leucocephala* (Lam.) de Wit subsp. *leucocephala* (Fabaceae), which as a nitrogen-fixer can alter ecosystem function where it invades (Mello *et al.*, 2016). Seeds used in the experiments were collected fresh from mostly natural or restored populations between July 2020 and January 2021, with the exception of *Argemone glauca* (Nutt. ex Prain) Pope var. *glauca*, *Cyperus pennatiformis* Kük. var. *pennatiformis*, *Sesuvium portulacastrum* (L.) L. and *Solanum nelsonii* Dunal, which were collected from cultivated plants in botanical gardens. *Brighamia insignis* A. Gray is endangered, and seeds were collected from cultivated plants at the Chicago Botanic Garden in January 2020 and mailed to Kaua'i. For all species, seeds were removed from fruit manually by hand, and a subset was randomly selected for use in the experiments. All seeds were stored at ambient laboratory conditions (~20 °C; 55

TABLE 1. Characteristics of all taxa included in salinity tolerance experiments.

Taxon	Family	Habit	Origin	Seed mass (mg)	Dormancy	Threatened
<i>Argemone glauca</i> (Nutt. ex Prain) Pope var. <i>glauca</i>	Papaveraceae	Forb	Endemic	2.64	MPD	NT
<i>Bacopa monnieri</i> (L.) Wettst.	Plantaginaceae	Forb	Indigenous	0.03	PD	NT
<i>Brighamia insignis</i> A. Gray	Campanulaceae	Tree	Endemic	0.20	MPD	T
<i>Chenopodium oahuense</i> (Meyen) Aellen	Amaranthaceae	Tree	Endemic	0.23	PD	NT
<i>Cyperus pennatiformis</i> Kük. var. <i>pennatiformis</i>	Cyperaceae	Graminoid	Endemic	0.06	PD	T
<i>Dodonaea viscosa</i> Jacq.	Sapindaceae	Tree	Indigenous	5.60	PY	NT
<i>Fimbristylis cymosa</i> R. Br.	Cyperaceae	Graminoid	Indigenous	0.08	PD	NT
<i>Gossypium tomentosum</i> Nutt. ex Seem.	Malvaceae	Shrub	Endemic	43.66	PY	T
<i>Heliotropium curassavicum</i> L.	Heliotropaceae	Forb	Indigenous	0.52	PD	NT
<i>Heteropogon contortus</i> (L.) P. Beauv. ex Roem. & Schult.	Poaceae	Graminoid	Indigenous	1.26	PD	NT
<i>Ipomoea pes-caprae</i> (L.) R. Br. subsp. <i>brasiliensis</i> (L.) Ooststr.*	Convolvulaceae	Vine	Indigenous	85.34	PY	NT
<i>Leucaena leucocephala</i> (Lam.) de Wit subsp. <i>leucocephala</i>	Fabaceae	Tree	Non-native	35.61	PY	NT
<i>Nama sandwicensis</i> A. Gray	Namaceae	Forb	Endemic	0.04	PD	NT
<i>Sesbania tomentosa</i> Hook. & Arn.	Fabaceae	Shrub	Endemic	21.81	PY	T
<i>Sesuvium portulacastrum</i> (L.) L.	Aizoaceae	Forb	Indigenous	0.30	PD	NT
<i>Solanum nelsonii</i> Dunal	Solanaceae	Shrub	Endemic	2.11	PD	T
<i>Thespesia populnea</i> (L.) Sol. ex Corrêa	Malvaceae	Tree	Indigenous	173.77	PY	NT
<i>Tridax procumbens</i> L.*	Asteraceae	Forb	Non-native	0.55	PD	NT
<i>Verbesina encelioides</i> (Cav.) Benth. & Hook.	Asteraceae	Forb	Non-native	1.50	PD	NT
<i>Vigna marina</i> (Burm.) Merr.	Fabaceae	Forb	Indigenous	72.31	PY	NT
<i>Waltheria indica</i> L.*	Malvaceae	Shrub	Indigenous	1.55	PD	NT

Habit = forb, graminoid, shrub, tree, vine. Distribution = endemic, indigenous, non-native. Dormancy: MPD = morphophysiological, PD = physiological, PY = physical. Threatened Status (combined federal conservation status and IUCN Red List status): T = Threatened, NT = Not Threatened.

*Taxa excluded from analyses due to low germination (<15 % germination across all replicates).

% relative humidity) until sown (between 28 and 356 d after collection; [Supplementary Data Table S1](#)).

Salinity treatments

Salinity treatments were imposed by incubating seeds directly in seawater collected from Lāwa'i Bay, Kaua'i, on 17 November 2020. We used seawater instead of the NaCl solutions more commonly used in similar studies as NaCl solutions do not simulate the effects of seawater in experimental trials ([Hanley et al., 2020b](#)). Total salinity of the collected seawater was measured by conductance at the SOEST Laboratory for Analytical Biogeochemistry (S-LAB) at the University of Hawai'i at Mānoa. Specific ion composition was measured by the Motzz Laboratory, Inc. in Phoenix, AZ, USA ([Supplementary Data Table S2](#)). The unfiltered, untreated and undiluted seawater (mean of three replicates = 34.75; hereafter 35 ppt) was used as the highest salinity treatment since this, under certain circumstances, would be the actual salinity level seeds would experience during coastal flooding or intrusion in their natural habitat. The unfiltered/untreated seawater was diluted with distilled water to obtain the other two salinity-level treatments (10 and 20 ppt total salinity). Distilled water was used to incubate the control group (0 ppt total salinity). This range of salinity concentrations includes levels below and above the typical threshold

of 20 ppt required by many halophytes for optimal performance ([Munns and Tester, 2008](#)).

Germination assays

Germination assays were conducted at the NTBG Seed Bank and Laboratory between December 2020 and March 2021. Five replicates of 20 seeds for each species (with the exception of *Sesbania tomentosa* Hook. & Arn., *Cyperus pennatiformis* var. *pennatiformis* and *Gossypium tomentosum* Nutt. ex Seem. with three replicates of 11, three replicates of 15 and five replicates of 18 seeds, respectively, due to difficulty in obtaining enough seeds) were randomly assigned to each of the three salinity and control groups (hereafter referred to as the 'salinity trial'). For taxa with reported or suspected seed dormancy, a dormancy breaking pre-treatment was carried out ([Supplementary Data Table S1](#)). Seeds were sown in 60-mm Petri dishes on seed germination paper (Anchor Paper Co., St. Paul, MN, USA) moistened with 1.75 mL of assigned salinity treatment solution or distilled water, sealed with plastic paraffin film to increase water retention, and placed in a germination chamber (Percival GR36L) in haphazard positions at a daily alternating 12-h light [$\sim 41 \mu\text{mol m}^{-2} \text{s}^{-1}$ cool white (4100 K) fluorescent light]/12-h dark photoperiod and 25/15 °C temperature regime. Throughout the salinity trial, distilled water was added as needed to maintain an even saturation of

seed germination paper. Germination (indicated by radicle emergence) was monitored 2 d per week for 13 weeks or until germination ceased across all replicates and treatments for a species for at least 7 d (whichever occurred first), after which the seeds were transferred to the recovery trial. In the case that at least 10 % of the seeds in the control treatment did not germinate, the salinity trial was concluded after 8 weeks and the seeds were transferred to the recovery trial.

To test for recovery germination, non-germinated seeds were rinsed with distilled water and transferred to clean Petri dishes with new seed germination paper moistened with distilled water only. Seeds in the recovery trial were monitored 2 d per week for at least 4 weeks or until germination ceased across all replicates and treatments for a species for at least 7 d. Recovery trials were ended early if all remaining seeds either germinated or died (due to fungal infection). Throughout the recovery trial, distilled water was added as needed to maintain an even saturation of seed germination paper.

For the duration of the experiments, the Petri dishes in the germination chamber were haphazardly re-positioned each time dishes were monitored for germination. Once the recovery trial was complete, remaining seeds were excised and embryos examined to determine viability (viable seeds determined by the presence of firm white embryos). Germination of federally listed Threatened and Endangered species was carried out under Hawai'i State Department of Land and Natural Resources, Natural Area Reserve, Rare Plant, and Native Invertebrate Research Permit number I2463. Resulting seedlings of all native species were transferred to the NTBG Conservation and Horticulture Center nursery.

Statistical analysis

Germination percent, timing and recovery were quantified and compared among species and across salinity levels. All analyses were done in the software environment R version 4.1.2. (R Core Team, 2021) and RStudio version 2022.7.1.554 (RStudio Team, 2022). Visualization was carried out using the R extension package 'ggplot2' (Wickham, 2016). Germination percent and timing were analysed using germination indices from the 'GerminAR' package (Lozano-Isla et al., 2019). Germination timing was calculated using mean germination time (MGT) which denotes the number of seeds germinated with respect to the number of seeds germinated at the time of evaluation. Species that had <15 % germination across all replicates were excluded from analyses, which resulted in the exclusion of *Ipomoea pes-caprae* (L.) R. Br. subsp. *brasiliensis* (L.) Ooststr., *Tridax procumbens* L. and *Waltheria indica* L. The statistical analyses thus included only 18 species. Furthermore, in 8 % of Petri dishes there were unresolved data entry errors, and these replicates were excluded from analyses, resulting in three to five Petri-dish replicates per species × treatment group. Recovery germination percentage (RGP) was calculated as $RGP = [a/(c - b)] \times 100$, where *a* is the total number of seeds germinated after transfer, *b* is the total number of seeds germinated under salinity treatment and *c* is the total seeds sown for each dish (Lum and Barton, 2020; Pujol et al., 2000).

Because our main goal was to determine whether species differed in their germination responses to salinity for insights into community-scale resilience to sea level rise, we analysed

species responses collectively using meta-analysis. Meta-analysis is commonly used within studies to synthesize results across experimental units (sites or species), and is particularly useful for identifying sources of heterogeneity among groups (Nakagawa and Cuthill, 2007; Gurevitch, 2013; Fajardo and Siefert, 2018; Barton and Shiels, 2020). Effect sizes were calculated for each salinity treatment level within species as log-response ratios: $\ln(M_s/M_c)$, where M_s is the mean germination of salinity treatment groups and M_c is the mean germination of the freshwater control groups. To avoid calculation errors resulting from zero germination scores, all replicates were adjusted with an increase of 0.01. Positive log response ratios indicated higher germination percent under salinity than control conditions, as would be predicted for halophytes, and negative log response ratios indicated reduced germination under salinity compared to freshwater conditions. Effect sizes were calculated for total germination (%) and mean time to germination (d). For recovery germination percent, means and standard deviations were used in place of log-response ratios because there are no recovery germination data for control groups, preventing us from calculating this effect size.

Our study examined a diverse species pool, reflective of coastal communities in Hawai'i, and we initially examined evidence for a phylogenetic signal in germination responses. A tree of our focal species (Supplementary Data Fig. S1) was pruned from a megacalibrated time-tree (Magallón et al., 2015; Table S1) and used to calculate Blomberg's *K* and Pagel's λ with the phylog function in the 'phytools' package (Revell, 2012). Blomberg's *K* and Pagel's λ test whether related species are more similar in salinity tolerance than would be expected under a Brownian model of evolution (Pagel, 1999; Blomberg and Garland, 2002; Münkemüller et al., 2012). For the three metrics, we found weak evidence for a phylogenetic signal in seed salinity tolerance (Table S3), and so a non-phylogenetic meta-analysis was conducted.

The meta-analysis was conducted using the 'metafor' package (Viechtbauer, 2014). Variation in germination responses among salinity treatment groups (10, 20, 35 ppt) was analysed with mixed-model analyses using the *rma.mv* function. Potential sources of heterogeneity among species were tested as fixed factors: seed dormancy class (physical, physiological, morphophysiological; determined by or inferred from Baldos et al., 2014; Baskin and Baskin, 2014; Wolkis et al., 2022), habit (graminoid, forb, shrub, tree), seed mass (Table 1) and threatened status (combined federal status and IUCN Red List threatened status, assigned as either threatened or not threatened; Supplementary Data Table S1). Because we had no a priori predictions about potential interactions among the fixed predictor variables, and because of non-independence among some of them (habit and threatened status: $\chi^2 = 12.18$, d.f. = 4, $P = 0.0161$), we ran separate univariate models for each predictor variable. In all of these mixed models, species was included as a random factor because there were three effect sizes analysed for each species (representing the responses to the three levels of salinity). To explore the interaction between seed mass and salinity, mixed-model analyses using the *rma.mv* function were performed with and without the interaction, and the models were compared using likelihood ratio tests (function *anova*). The interaction effect was visualized with the package 'sjPlot' (Lüdtke, 2023) with function *plot_model*.

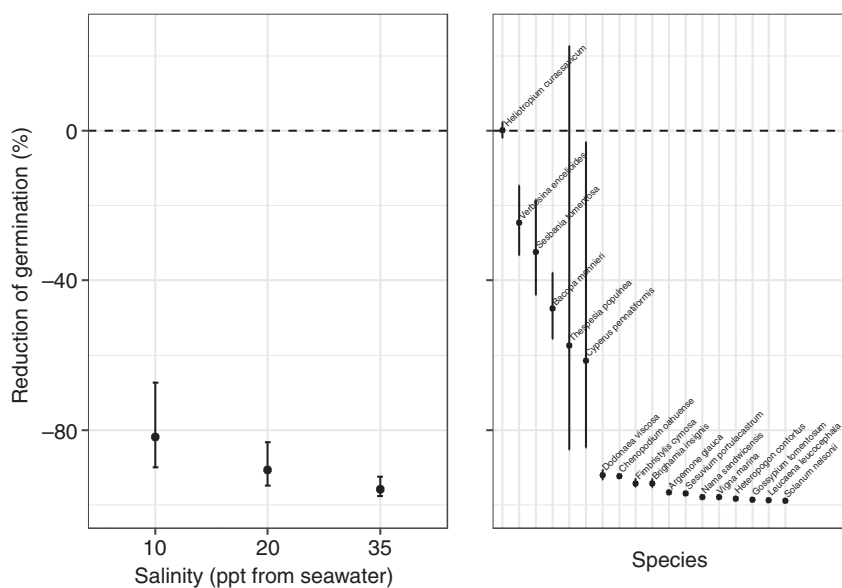


FIG. 1. Percent reduction in germination after the salinity trial compared to germination in distilled water control. The left panel is all 18 species included in analyses at each salinity level treatment (10, 20 and 35 ppt from seawater) and the right panel is each of the 18 species separately across all salinity treatments. To facilitate interpretation, log response ratios were back-transformed to percentage differences between salinity and control groups as: $100^{(\text{response ratio})} - 100$. Model-generated group means and 95 % confidence intervals are presented. When confidence intervals do not overlap with zero, this indicates a significant effect of salinity on germination.

To further characterize community-level patterns, we categorized the salinity responses of each species according to Boorman's (1968) classification system of Group 1, 2 and 3 species and recognized a fourth group (Group 4) as described by Hanslin and Eggen (2005). We used ANOVA and post-hoc Tukey tests to compare mean germination percent after the salinity trial (Supplementary Data Table S4) and total mean germination after the recovery trial to mean germination in the control for each species individually to assign as either a Group 1, 2, 3 or 4 species. Group 1 species have low germination under salinity that is inversely proportional to the salinity concentration, and recovery by freshwater is incomplete, revealing these species to be sensitive to both the osmotic and toxic effects of salinity. Group 2 species germinate at low salinity, and although suppressed at high salinity, they achieve high recovery germination, indicating minimal toxicity and a predominantly osmotic effect under high salinity. Group 3 species are either unaffected or induced by high salinity, to achieve greater germination than seeds experiencing only freshwater conditions. Group 3 species are generally halophytes restricted to habitats with chronically high salinity such as salt marshes, while Groups 1 and 2 have been described for species in all coastal ecosystems (Woodell, 1985). Group 4 species have been described to include coastal plants with nearly complete failure to germinate under salinity and minimal recovery, indicating very high sensitivity to salinity leading to seed mortality (Hanslin and Eggen, 2005). Classifying coastal plants in this way may facilitate predictions about the stability and future composition of coastal ecosystems under sea level rise and elevated salinity. For example, a community composed largely of species of Groups 1 and 2 would be expected to undergo dramatic shifts in community assembly as most species would decline under elevated salinity. On the other hand,

a community dominated by species of Group 3 would be predicted to have greater stability.

RESULTS

Germination responses to salinity

Across all taxa and salinity treatment groups, mean germination ranged from 0 to 98 %. Overall, salinity significantly reduced germination compared to freshwater control conditions ($Z = -8.3414$, $P < 0.001$). Salinity effects were dosage-dependent, with increasingly fewer seeds germinating under higher salinity levels ($Q_M = 3003.64$, d.f. = 2, $P < 0.0001$, Fig. 1). Considerable variability was detected among species ($Q_M = 189\,443.57$, d.f. = 18, $P < 0.0001$, Fig. 1; Supplementary Data Fig. S2). Seed mass predicted mean germination, but this effect varied significantly among levels of salinity (likelihood ratio test of interaction term: $\chi^2 = 546.67$, d.f. = 5, $P < 0.0001$). In general, we detected higher germination under salinity exposure for larger seeded species. Specifically, there was no apparent relationship between seed mass and germination under the 10 ppt treatment, but under the 20 and 35 ppt salinity-level treatments, species with larger seeds had higher mean germination (Fig. 2A). We did not detect evidence that germination during salinity exposure depends on threatened status ($Q_M = 0.0096$, d.f. = 1, $P = 0.9220$), habit ($Q_M = 0.5369$, d.f. = 3, $P = 0.9046$) or seed dormancy class ($Q_M = 0.5100$, d.f. = 2, $P = 0.7749$).

Mean germination time was also highly variable, ranging from 5 d [*Verbesina encelioides* (Cav.) Benth. & Hook., 0 ppt] to 71 d [*Bacopa monnieri* (L.) Wettst., 20 ppt]. Overall, salinity delayed germination compared to freshwater control

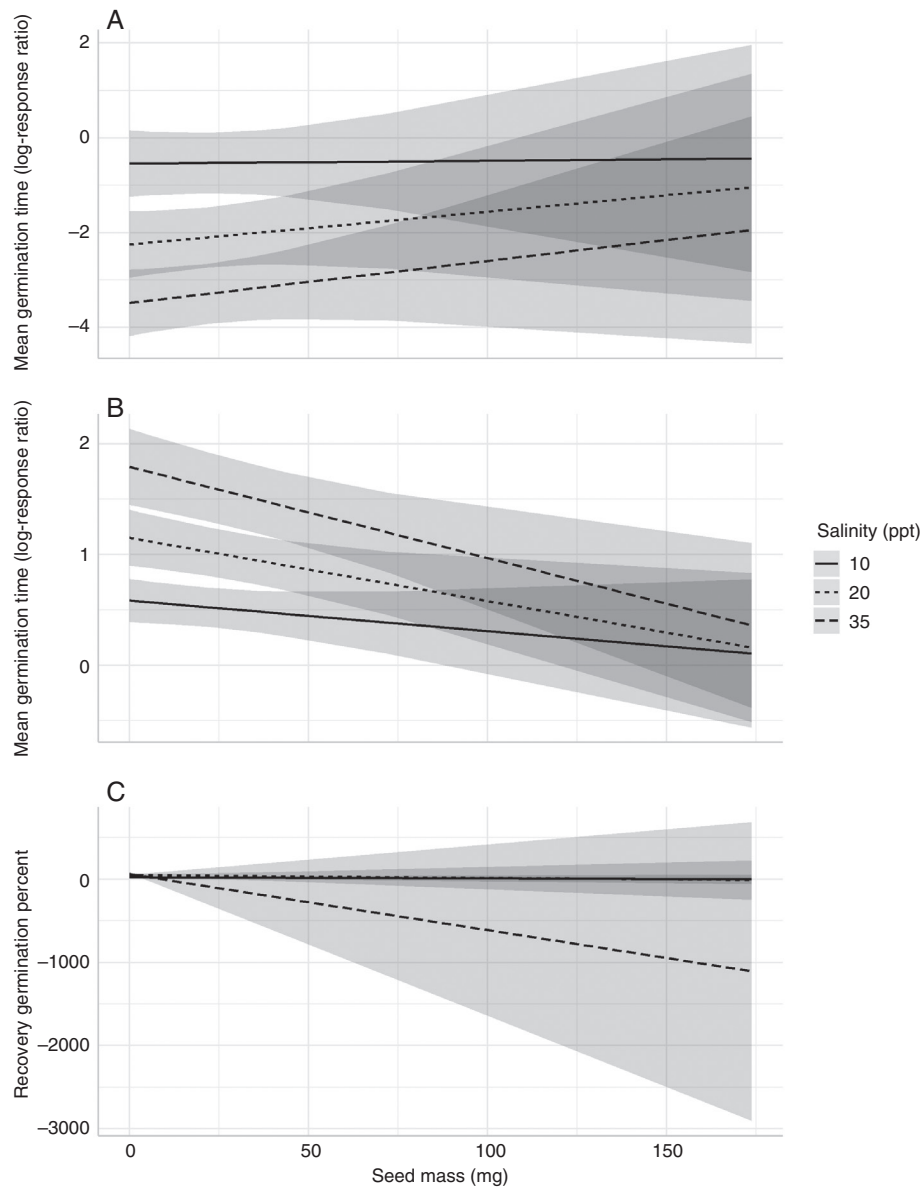


FIG. 2. Interaction effects between seed mass (mg) and (A) mean germination percent, (B) mean time to germination and (C) recovery germination percent at the three salinity treatments (10, 20 and 35 ppt seawater). The interaction visualized was the result of a linear model and graphed with the R package 'sjPlot' (Lüdtke, 2023) using the function plot_model.

conditions ($Z = 7.1306$, $P < 0.0001$). Salinity effects were dosage-dependent, with increasingly longer germination delays under higher salinity levels ($Q_M = 386.2302$, d.f. = 2, $P < 0.0001$, Fig. 3). As with mean germination, significant variability was detected among species in germination time ($Q_M = 1555.3990$, d.f. = 15, $P < 0.0001$, Fig. 3; Supplementary Data Fig. S3), and none of the heterogeneity tests conducted could explain this variability: threatened status ($Q_M = 0.4954$, d.f. = 1, $P = 0.4815$), habit ($Q_M = 0.2858$, d.f. = 3, $P = 0.9627$) or seed dormancy class ($Q_M = 2.8542$, d.f. = 2, $P = 0.2400$). Seed mass, however, predicted mean germination time and this effect varied significantly among levels of salinity (likelihood ratio test of interaction term: $\chi^2 = 12.2018$, d.f. = 5,

$P = 0.0022$). In general, larger seeded species appeared to germinate faster under salinity compared to species with smaller seeds, with the strongest relationship emerging under high and intermediate salinity levels (Fig. 2B).

Recovery germination

Recovery germination under freshwater conditions varied significantly among species ($Z = 8.1777$, $P < 0.001$, Fig. 4). Significantly higher recovery germination percentages were observed for seeds exposed to high salinity ($Q_M = 1125.4970$, d.f. = 2, $P < 0.001$, Fig. 4), suggesting that 20 and 35 ppt salinity treatments were not lethal to seeds for most species,

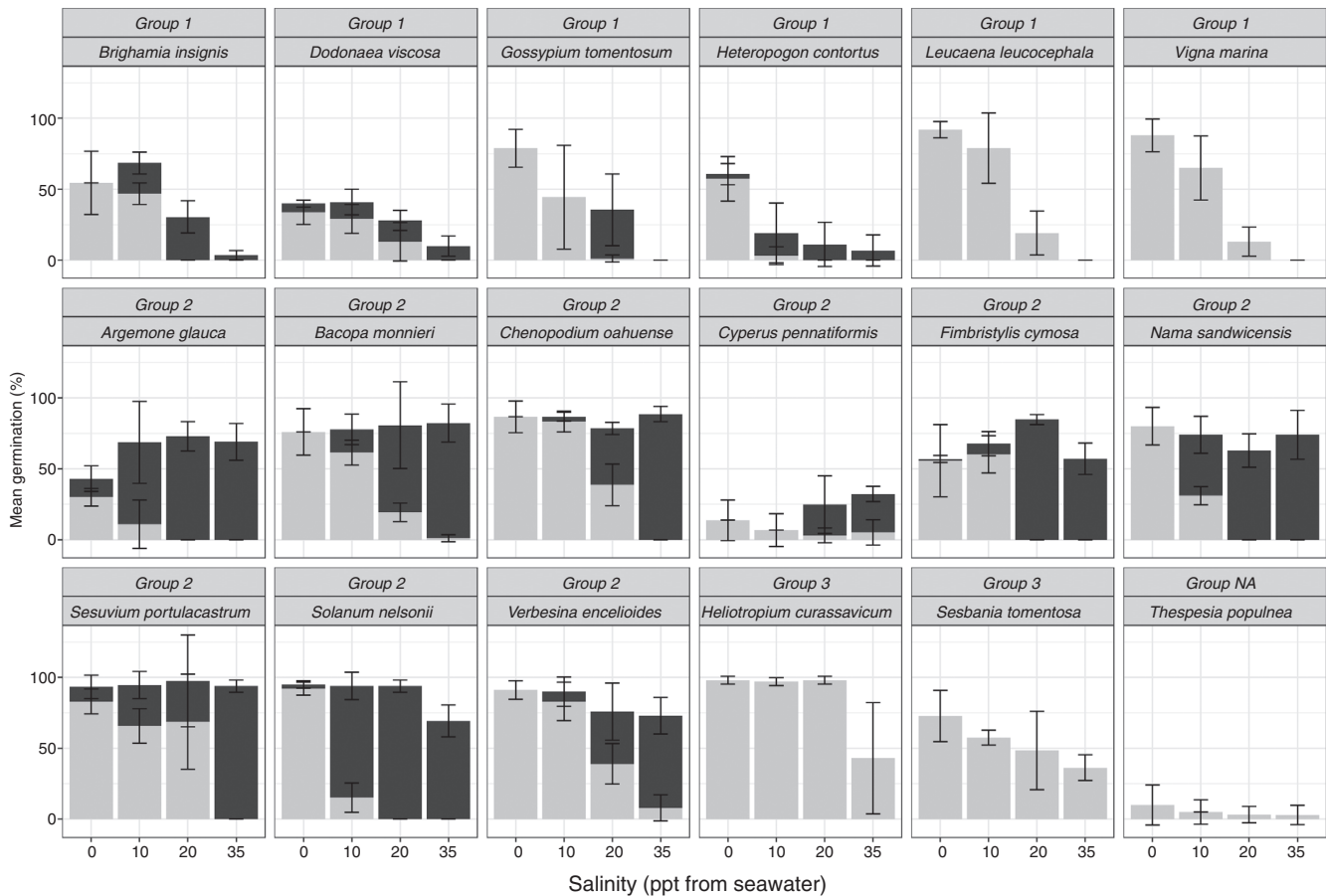


Fig. 5. Mean germination percent after the salinity trial (grey bars) and recovery trial (black bars) for each of the 18 species included in analyses at the control (0 ppt) and three salinity level treatments (10, 20 and 35 ppt from seawater). Error bars are means \pm 1 s.d. Species are labelled with and ordered by group assignment (Group 1, 2 or 3) as defined by Boorman (1968) from left to right starting with the top row and within each group, in alphabetical order.

$P = 0.6736$) or seed dormancy class ($Q_M = 2.6549$, d.f. = 2, $P = 0.2652$). However, recovery germination was found to differ significantly among habit ($Q_M = 10.8598$, d.f. = 3, $P < 0.0125$), with higher recovery germination in forbs and shrubs compared to graminoids and trees (Fig. 6). Seed mass also predicted recovery germination and this effect varied significantly among levels of salinity as it did for mean germination and mean time to germination (likelihood ratio test of interaction term: $\chi^2 = 129.15$, d.f. = 5, $P < 0.0001$). Specifically, there was no apparent relationship between seed mass and recovery germination under the 10 and 20 ppt treatments, but under the 35 ppt salinity level treatment, species with larger seeds had significantly lower recovery germination (Fig. 2C).

In total, 33 % of species were classified as Group 1 species, with low germination during salinity exposure and recovery. Fifty per cent of the species were classified as Group 2. These species were able to germinate at low salinity as well as achieved high recovery germination. Eleven per cent were categorized as Group 3 species. In this case, germination was unaffected by high salinity. There were no Group 4 species classified. A single species had low germination overall, including the control, and therefore was not classified (Fig. 5).

DISCUSSION

Across the 18 Hawaiian coastal plant taxa investigated here, we found that salinity exposure reduced and delayed germination overall, with the greatest effects detected at the higher salinity treatment levels. While recovery germination was higher for seeds that had been exposed to higher salinity treatments, this was largely driven by higher germination at the lower salinity treatments and control.

The overall pattern of reduced and delayed germination under high salinity is consistent with responses in other coastal species (Necajeva and Ievinsh, 2008; Santo *et al.*, 2017; Del Vecchio *et al.*, 2020; Lum and Barton, 2020; Moreno *et al.*, 2022). However, most of these studies used NaCl solutions, which Hanley *et al.* (2020b) demonstrated experimentally are unsuitable surrogates for studying the effect of seawater on plant physiology, and so may not accurately reflect germination responses to the full suite of salt ions found in seawater. For the majority of species in our study, nearly 67 % (12 out of 18 included in analyses), we detected a dramatic reduction in germination of >90 % under salinity (Fig. 1), which is a greater reduction than is commonly found for coastal plants. For example, Del Vecchio *et al.* (2020) found that the least tolerant species in their community-scale study had a >80 % reduction

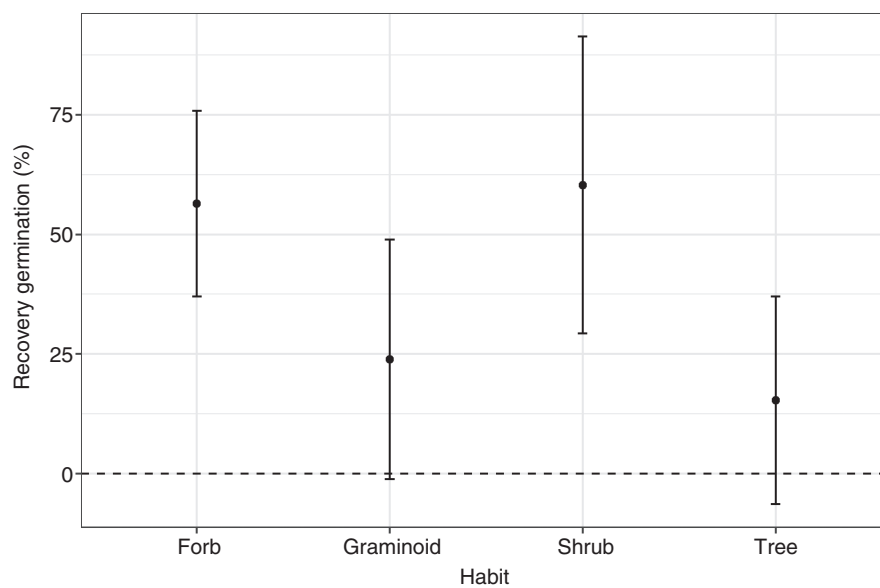


FIG. 6. Mean germination percent after the recovery trial with all 18 species included in analyses grouped by habit. Error bars are lower and upper confidence intervals.

in germination. Inhibited germination after exposure to full salinity seawater, or its approximate salinity equivalent in a NaCl solution, has been found in other species elsewhere, including species from some of the same plant families (Asteraceae, Chenopodiaceae, Fabaceae and Poaceae) for which we had representative species with completely inhibited germination (Martínez *et al.*, 1992; Houle *et al.*, 2001; Muñoz-Rodríguez *et al.*, 2017; Malik *et al.*, 2022). Some populations of Hawaiian coastal species are already experiencing complete inundation from seawater with the combined effects of sea level rise, high tides and large wave swells. Reduced germination could be a major bottleneck for population persistence for many Hawaiian coastal plant species in the near future as sea levels rise, even greater than has been reported in other regions (Del Vecchio *et al.*, 2020). Furthermore, shifts in the timing of germination could alter species interactions due to size asymmetries emerging among seedlings of different species that germinate at different times, potentially altering competition and overall community composition (Heard and Ancheta, 2011). Characterizing the effects of germination responses on subsequent species interactions warrants additional research to more fully predict the effects of sea level rise on community dynamics.

Recovery germination is a common response of coastal plants, allowing them to withstand ephemeral periods of high salinity. Although recovery germination percent was overall higher for seeds that had been exposed to higher salinity level treatments, this was variable across our focal species. Others have also found high variability in recovery germination across species (Martínez *et al.*, 1992; Houle *et al.*, 2001; Necajeva and Levinsh, 2008; Guja *et al.*, 2010; Bhatt and Santo, 2016; Santo *et al.*, 2017; Lum and Barton, 2020; Moreno *et al.*, 2022). Low recovery germination has previously been detected following 20 ppt treatment in two other native Hawaiian coastal plant species that we did not include in our study (*Jacquemontia sandwicensis* and *Sida fallax*; Lum and Barton, 2020). While recovery germination is likely to have evolved in response to

short-term elevated salinity, reduced precipitation could make these responses ineffective if the periods of low salinity fail to occur. Projected climatic changes would probably alter normal regeneration patterns for the majority of the species in our study and thus the species composition and diversity of Hawaiian coastal communities. Since only filled seeds were considered in our study (all remaining seeds after experiments ended were excised to determine whether they contained an embryo or were empty), we suspect that the seeds of the species that were unable to recover were killed by salinity exposure in the salinity trials, probably due to ion toxicity.

Because sea level rise will eventually lead to habitat loss, coastal plant persistence will ultimately rely on population migration inland to suitable habitats. The biological processes underlying population stability in the short term and population migration in the long term are poorly studied in coastal dune plants in particular, with little information about dispersibility, phenotypic plasticity or rapid evolution. In addition, the potential role of epigenetics by which maternal plants may influence the salinity tolerance of offspring remains poorly studied, although this has emerged as a potential factor enhancing halophyte performance. Guo *et al.* (2020) found, for example, that seeds collected from mother plants of the extreme halophyte *Suaeda salsa* that were exposed to higher salinity levels subsequently had higher germination compared to seeds collected from mother plants grown in control and lower salinity conditions. The trends we observed could vary due to epigenetic effects depending on the populations and/or specific mother plants from which seeds were collected, especially considering seeds for almost all of the species in our study were collected from cultivated or outplanted individuals and seed collections from different mother plants were aggregated for the experiments (Supplementary Data Table S1). Future studies tracking maternal environments would help elucidate this process in coastal plant community salinity tolerance. Other environmentally driven variability at the individual or population scale

(Ghars *et al.*, 2009; Murru *et al.*, 2015), including phenotypic plasticity and genetic differentiation, should also be priorities for a better understanding of the evolutionary dynamics of coastal plant responses to sea level rise.

Of the factors we examined as possible predictors of salinity tolerance among species, only seed mass emerged as a predictor for all three response variables (mean germination percent, mean time to germination and recovery germination). Under moderate and high salinity levels, larger seeded species had higher mean germination, which indicates a benefit of large seeds under elevated salinity. Under any level of salinity exposure, species with larger seeds germinate faster. Easton and Kleindorfer (2009) also found that species with larger seeds had higher germination and germination time at higher salinity levels among the 12 Australian species of *Frankenia* that they investigated. That seed size relates to salinity tolerance during germination is not surprising, given that seed size variation is a fundamental axis of plant function and stress tolerance (Leishman *et al.*, 2000; Díaz *et al.*, 2016), and future studies linking germination responses under elevated salinity to seedling performance and establishment would provide additional insights into these effects (Moles and Westoby, 2004).

Plant habit also emerged as a significant predictor for recovery germination, with forbs and shrubs having higher recovery germination compared to graminoids and trees (Fig. 6). This could be linked to seed size variation among these groups, or perhaps is merely a consequence of the relatively small number of species included in the analysis. Variability among forms has previously been detected in an arctic ecosystem, with sites categorized as graminoid and upright shrub sites having higher recovery after saline incursion compared to sites categorized as dwarf shrub-dominated (Lantz *et al.*, 2015).

Because threatened status did not emerge as a predictor of salinity tolerance, we do not suspect that sea level rise and increased drought were the major factors that led to the initial decline of the threatened coastal species included our study. Non-native species and human use of coastal areas were previously identified as the greatest threats to native coastal vegetation in Hawai'i (Warshauer *et al.*, 2009). Three of the threatened species in our study have been federally listed as Endangered since 1994 (*Brighamia insignis*, *Cyperus pennatifolius* and *Sesbania tomentosa*; USFWS, 2023). Although sea levels have been rising in Hawai'i for over a century, rates of rise continue to accelerate (Eversole, 2014), which will probably result in sea level rise becoming one of the major threats to the already threatened species and negatively impact other species as well.

Interestingly, the three non-native species we included in our study had quite different responses. One of them was excluded from analyses due to low germination overall (*Tridax procumbens*). The two others responded quite differently, with *L. leucocephala* having incomplete recovery from salinity exposure whereas *Verbesina encelioides* had high recovery germination. There is thus also variability in salinity tolerance among non-native coastal species that occur in Hawai'i as we found for the native species we included. Although there are also many other factors and traits that result in an introduced plant species becoming invasive, research examining species-specific responses to salinity of more non-native species at the seed germination and other life stages is warranted, as well

as exploring potential differences in salinity tolerance of native versus non-native species. Greater salinity tolerance of non-native compared to native plants may lead to expansion of the non-native species, resulting in declines in native species. We did not explore species origin as a predictor of salinity tolerance due to the small number of non-native species we included in our study.

Although seed dormancy class did not explain any of the differences we observed in salinity tolerance, our experimental design did not allow us to truly examine this since we conducted dormancy breaking pretreatments on all the species with known dormancy prior to sowing, which were all of the species (Baldos *et al.*, 2014; Baskin and Baskin, 2014; Wolkis *et al.*, 2022; Table 1; Supplementary Data Table S1). We suspect we would have observed differences in germination responses to salinity if we had not conducted dormancy breaking pretreatments prior to sowing seeds in experimental treatments.

We synthesized results of the salinity trials and recovery trials to characterize each species into Groups 1, 2, 3 or 4 type species with regard to salinity tolerance (Boorman, 1968; Hanslin and Eggen, 2005). We found that the majority of the taxa (83 %) can be classified as Group 1 or 2 species, with low germination during salinity exposure and recovery or the ability to germinate at low salinity as well as achieved high recovery germination, respectively. Woodell (1985) assessed seed salinity tolerance in many species from a variety of coastal habitats including dunes, salt marsh, shingle and driftline and found that most dune species were the least tolerant and thus were assigned as Group 1 species. One of the most commonly used species in coastal restoration projects, *Dodonaea viscosa* Jacq., was classified as a Group 1 species, with low germination during salinity exposure and recovery. No seeds germinated after initial exposure to the 35 ppt salinity level treatment in the salinity trial and only 10 % were able to recover from exposure in that treatment. Results such as this could be interpreted from a restoration management perspective as perhaps deciding to utilize *D. viscosa* in coastal restoration areas further inland. Research to enhance *D. viscosa* performance under elevated salinity has been conducted in many other parts of the world (Yousefi *et al.*, 2017), highlighting the value of these approaches to inform restoration.

Our community perspective of species salinity tolerance at the early life stage of seed germination suggests that, overall, coastal ecosystems in Hawai'i are highly vulnerable to increases in salinity exposure. Recruitment bottlenecks for many species will probably occur as sea levels rise, leading to changes in community assembly and composition (Hanley *et al.*, 2020a). Research examining salinity tolerance at the other life stages is also needed, which has been found to vary among two other Hawaiian coastal species (Lum and Barton, 2020) and co-occurring coastal species elsewhere (Hanley *et al.*, 2017, 2020b). Experimental research testing the combined effects of other co-occurring stressors such as drought and temperature is also warranted (Heard and Ancheta, 2011; Santo *et al.*, 2014; Del Vecchio *et al.*, 2020). Applied experimental research such as this will be one way forward in determining the best adaptation strategies for coastal ecosystem conservation and management in the face of climate change.

SUPPLEMENTARY DATA

Supplementary data are available at *Annals of Botany* online and consist of the following.

Table S1. Specific methods related to seed collection, germination trials, conservation status and phylogenetic analysis for each of the 21 species included in the study. Table S2. Specific ion composition of unfiltered, untreated and undiluted seawater collected from Lāwa'i Bay, Kaua'i, on 17 November 2020. Table S3. Bloomberg's *K*, Pagel's λ and *P*-values for germination percent and mean time to germination after the salinity trial and recovery germination percent after the recovery trial at each of the three salinity level treatments. Table S4. For each of the 18 species used in analyses, ANOVA and treatment pairwise post-hoc Tukey test *P*-values after the salinity trial. Fig. S1. Phylogenetic tree of the 18 species used in the phylogenetic correlation analysis. Fig. S2. Germination percent after the salinity trial for each of the 18 species included in analyses at the control and three salinity level treatments. Fig. S3. Mean germination time in days for each of the 18 species included in analyses at the control and three salinity level treatments.

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LITERATURE CITED

- Alpha CG, Drake DR, Goldstein G. 1996. Morphological and physiological responses of *Scaevola sericea* (Goodeniaceae) seedlings to salt spray and substrate salinity. *American Journal of Botany* **83**: 86–92. doi:10.2307/2445958.
- Anderson TR, Fletcher CH, Barbee MM, Romine BM, Lemmo S, Delevaux JMS. 2018. Modeling multiple sea level rise stresses reveals up to twice the land at risk compared to strictly passive flooding methods. *Scientific Reports* **8**: 14484. doi:10.1038/s41598-018-32658-x.
- Baldos OC, DeFrank J, Kramer M, Sakamoto GS. 2014. Storage humidity and temperature affect dormancy loss and viability of tanglehead (*Heteropogon contortus*) seeds. *HortScience* **49**: 1328–1334. doi:10.21273/hortsci.49.10.1328.
- Barton KE, Shiels AB. 2020. Additive and non-additive responses of seedlings to simulated herbivory and drought. *Biotropica* **52**: 1217–1228. doi:10.1111/btp.12829.
- Baskin CC, Baskin JM. 2014. *Seeds: ecology, biogeography, and evolution of dormancy and germination*, 2nd edn. San Diego: Elsevier.
- Bhatt A, Santo A. 2016. Germination and recovery of heteromorphic seeds of *Atriplex canescens* (Amaranthaceae) under increasing salinity. *Plant Ecology* **217**: 1069–1079. doi:10.1007/s11258-016-0633-6.
- Blomberg SP, Garland T Jr. 2002. Tempo and mode in evolution: phylogenetic inertia, adaptation and comparative methods. *Journal of Evolutionary Biology* **15**: 899–910. doi:10.1046/j.1420-9101.2002.00472.x.
- Boorman LA. 1968. Some aspects of the reproductive biology of *Limonium vulgare* Mill., and *Limonium humile* Mill. *Annals of Botany* **32**: 803–824. doi:10.1093/oxfordjournals.aob.a084251.
- Burke L, Kura Y, Kassem K, Revenga C, Spalding M, McAllister D. 2001. *Pilot analysis of global ecosystems: coastal ecosystems*. Washington, DC: World Resources Institute.
- Dasgupta S, Hossain MM, Huq M, Wheeler D. 2015. Climate change and soil salinity: The case of coastal Bangladesh. *Ambio* **44**: 815–826. doi:10.1007/s13280-015-0681-5.
- Debez A, Braun H-P, Pich A, et al. 2012. Proteomic and physiological responses of the halophyte *Cakile maritima* to moderate salinity at the germinative and vegetative stages. *Journal of Proteomics* **75**: 5667–5694. doi:10.1016/j.jprot.2012.08.012.
- Del Vecchio S, Porceddu M, Fantinato E, Acosta ATR, Buffa G, Bacchetta G. 2018. Germination responses of Mediterranean populations of *Cakile maritima* to light, salinity and temperature. *Folia Geobotanica* **53**: 417–428. doi:10.1007/s12224-018-9332-5.
- Del Vecchio S, Fantinato E, Roschini M, Acosta ATR, Bacchetta G, Buffa G. 2020. The germination niche of coastal dune species as related to their occurrence along a sea–inland gradient. *Journal of Vegetation Science* **31**: 1112–1121.
- Díaz S, Kattge J, Cornelissen JHC, et al. 2016. The global spectrum of plant form and function. *Nature* **529**: 167–171. doi:10.1038/nature16489.
- Easton LC, Kleindorfer S. 2009. Effects of salinity levels and seed mass on germination in Australian species of *Frankenia* L. (Frankeniaceae). *Environmental and Experimental Botany* **65**: 345–352. doi:10.1016/j.envexpbot.2008.10.006.
- Elison Timm O, Giambelluca TW, Diaz HF. 2015. Statistical downscaling of rainfall changes in Hawai'i based on the CMIP5 global model projections. *Journal of Geophysical Research: Atmospheres* **120**: 92–112.
- El-Katony TM, Khedr A-H A-F, Soliman NG. 2015. Nutrients alleviate the deleterious effect of salinity on germination and early seedling growth of the psammophytic grass *Elymus farctus*. *Botany* **93**: 559–571.
- Eversole D. 2014. *Climate change impacts in Hawai'i - A summary of climate change and its impacts to Hawai'i's ecosystems and communities*. Honolulu, Hawai'i: University of Hawai'i at Mānoa Sea Grant College Program. <https://repository.library.noaa.gov/view/noaa/39931>.
- Fajardo A, Siefert A. 2018. Intraspecific trait variation and the leaf economics spectrum across resource gradients and levels of organization. *Ecology* **99**: 1024–1030. doi:10.1002/ecy.2194.
- Fernández-Palacios JM, Kreft H, Irl SDH, et al. 2021. Scientists' warning – the outstanding biodiversity of islands is in peril. *Global Ecology and Conservation* **31**: e01847. doi:10.1016/j.gecco.2021.e01847.
- Ghars MA, Debez A, Abdelly C. 2009. Interaction between salinity and original habitat during germination of the annual seashore halophyte *Cakile maritima*. *Communication in Soil and Plant Analysis* **40**: 3170–3180.
- Goldstein G, Drake DR, Alpha C, Melcher P, Heraux J, Azocar A. 1996. Growth and photosynthetic responses of *Scaevola sericea*, a Hawaiian coastal shrub, to substrate salinity and salt spray. *International Journal of Plant Sciences* **157**: 171–179. doi:10.1086/297336.
- Guja LK, Merritt DJ, Kingsley WD. 2010. Buoyancy, salt tolerance and germination of coastal seeds: Implications for oceanic hydrochorous dispersal. *Functional Plant Biology* **37**: 1175–1186.
- Guja L, Wührer R, Moran K, Dixon KW, Wardell-Johnson G, Merritt DJ. 2013. Full spectrum X-ray mapping reveals differential localization of salt

- in germinating seeds of differing salt tolerance. *Botanical Journal of the Linnean Society* **173**: 129–142. doi:10.1111/boj.12072.
- Guo J, Du M, Tian H, Wang B. 2020. Exposure to high salinity during seed development markedly enhances seedling emergence and fitness of the progeny of the extreme halophyte *Suaeda salsa*. *Frontiers in Plant Science* **11**: 1291. doi:10.3389/fpls.2020.01291.
- Gurevitch J. 2013. Meta-analysis of results from multisite studies. In: Koricheva J, Gurevitch J, Mengersen K, eds. *Handbook of meta-analysis in ecology and evolution*. Princeton: Princeton University Press, 313–320.
- Hanley ME, Gove TL, Cawthray GR, Colmer TD. 2017. Differential responses of three coastal grassland species to seawater flooding. *Journal of Plant Ecology* **10**: rtw037–rtw330. doi:10.1093/jpe/rtw037.
- Hanley ME, Bouma TJ, Mossman HL. 2020a. The gathering storm: optimizing management of coastal ecosystems in the face of a climate-driven threat. *Annals of Botany* **125**: 197–212.
- Hanley ME, Sanders SKD, Stanton H-M, Billington RA, Boden R. 2020b. A pinch of salt: response of coastal grassland plants to simulated seawater inundation treatments. *Annals of Botany* **125**: 265–276.
- Hanslin HM, Eggen T. 2005. Salinity tolerance during germination of seashore halophytes and salt-tolerant grass cultivars. *Seed Science Research* **15**: 43–50. doi:10.1079/ssr.2004196.
- Harper JL. 1977. *Population biology of plants*. New York: Academic Press.
- Heard SB, Ancheta J. 2011. Effects of salinity and temperature on ex situ germination of the threatened Gulf of St. Lawrence Aster, *Symphotrichum laurentianum* Fernald (Nesom). *Plant Species Biology* **26**: 158–162. doi:10.1111/j.1442-1984.2011.00321.x.
- Houle G, Morel L, Reynolds CE, Siégel J. 2001. The effect of salinity on different developmental stages of an endemic annual plant, *Aster laurentianus* (Asteraceae). *American Journal of Botany* **88**: 62–67.
- Ignaciuk R, Lee JA. 1980. The germination of four annual strand-line species. *New Phytologist* **84**: 581–591. doi:10.1111/j.1469-8137.1980.tb04772.x.
- IPCC. 2021. Summary for policymakers. In: Masson-Delmotte V, Zhai P, Pirani A, et al. eds. *Climate change 2021: the physical science basis. Contribution of working group I to the sixth assessment report of the intergovernmental panel on climate change*. Cambridge: Cambridge University Press, 3–32.
- Kopp RE, Horton RM, Little CM, et al. 2014. Probabilistic 21st and 22nd century sea-level projections at a global network of tide-gauge sites. *Earths Future* **2**: 383–406.
- Lantz TC, Kokelj SV, Fraser RH. 2015. Ecological recovery in an Arctic delta following widespread saline incursion. *Ecological Applications* **25**: 172–185. doi:10.1890/14-0239.1.
- Leishman M, Wright I, Moles A, Westoby M. 2000. The evolutionary ecology of seed size. In: Fenner M, ed. *Seeds: the ecology of regeneration in plant communities*, 2nd edn. Wallingford: CAB International, 31–57.
- Llanes A, Andrade A, Masciarelli O, Alemanno S, Luna V. 2015. Drought and salinity alter endogenous hormonal profiles at the seed germination phase. *Seed Science Research* **26**: 1–13. doi:10.1017/s0960258515000331.
- Lozano-Isla F, Benites-Alfaro OE, Pompelli MF. 2019. GerminAR: An R package for germination analysis with the interactive web application ‘GerminaQuant for R’. *Ecological Research* **34**: 339–346.
- Lüdecke D. 2023. *sjPlot: Data Visualization for Statistics in Social Science*. R package version 2.8.14. <https://CRAN.R-project.org/package=sjPlot>.
- Lum TD, Barton KE. 2020. Ontogenetic variation in salinity tolerance and ecophysiology of coastal dune plants. *Annals of Botany* **125**: 301–314. doi:10.1093/aob/mcz097.
- Magallón S, Gómez-Acevedo S, Sánchez-Reyes LL, Hernández-Hernández T. 2015. A metacalibrated time-tree documents the early rise of flowering plant phylogenetic diversity. *New Phytologist* **207**: 437–453. doi:10.1111/nph.13264.
- Malik JA, Al-Qarawi AA, Al-Zain MN, Dar BA, Habib MM, Ibrahim SNS. 2022. Effect of salinity and temperature on the seed germination and seedling growth of desert forage grass *Lasiurus scindicus* Henr. *Sustainability* **14**: 8387. doi:10.3390/su14148387.
- Marra JJ, Merrifield MA, Sweet WV. 2012. Sea level and coastal inundation on Pacific islands. In: Keener VW, Marra JJ, Finucane ML, Spooner D, Smith MH, eds. *Climate change and Pacific islands: indicators and impacts. Report for the 2012 Pacific islands regional climate assessment (PIRCA)*. Washington, DC: Island Press, 65–88.
- Martínez ML, Valverde T, Moreno-Casasola P. 1992. Germination response to temperature, salinity, light and depth of sowing of ten tropical dune species. *Oecologia* **92**: 343–353. doi:10.1007/BF00317460.
- Martínez ML, Intralawan A, Vázquez G, Pérez-Maqueo O, Sutton P, Landgrave R. 2007. The coasts of our world: ecological, economic and social importance. *Ecological Economics* **63**: 254–272. doi:10.1016/j.ecolecon.2006.10.022.
- Mello TJ, Oliveira AA. 2016. Making a bad situation worse: an invasive species altering the balance of interactions between local species. *PLoS One* **11**: e0152070. doi:10.1371/journal.pone.0152070.
- Moles AT, Westoby M. 2004. Seedling survival and seed size: a synthesis of the literature. *Journal of Ecology* **92**: 372–383. doi:10.1111/j.0022-0477.2004.00884.x.
- Moreno J, Terrones A, Juan A. 2022. Germination patterns along a salinity gradient of closely-related halophytes in sympatry. *Estuarine, Coastal and Shelf Science* **264**: 107690. doi:10.1016/j.ecss.2021.107690.
- Münkemüller T, Lavergne S, Bzeznik B, et al. 2012. How to measure and test phylogenetic signal. *Methods in Ecology and Evolution* **3**: 743–756. doi:10.1111/j.2041-210x.2012.00196.x.
- Munns R, Tester M. 2008. Mechanisms of salinity tolerance. *Annual Review of Plant Biology* **59**: 651–681. doi:10.1146/annurev.arplant.59.032607.092911.
- Muñoz-Rodríguez AF, Sanjosé I, Márquez-García B, et al. 2017. Germination syndromes in response to salinity of Chenopodiaceae halophytes along the intertidal gradient. *Aquatic Botany* **139**: 48–56. doi:10.1016/j.aquabot.2017.02.003.
- Murru V, Santo A, Piazza C, Hugot L, Bacchetta G. 2015. Seed germination, salt-stress tolerance, and the effect of nitrate on three Tyrrhenian coastal species of the *Silene mollissima* aggregate (Caryophyllaceae). *Botany* **93**: 881–892. doi:10.1139/cjb-2015-0148.
- Nakagawa S, Cuthill IC. 2007. Effect size, confidence interval and statistical significance: a practical guide for biologists. *Biological Reviews* **82**: 591–605. doi:10.1111/j.1469-185X.2007.00027.x.
- Necajeva J, Ievinsh G. 2008. Seed germination of six coastal plant species of the Baltic region: effect of salinity and dormancy-breaking treatments. *Seed Science Research* **18**: 173–177. doi:10.1017/s0960258508040403.
- Pagel M. 1999. Inferring the historical patterns of biological evolution. *Nature* **401**: 877–884. doi:10.1038/44766.
- Pujol JA, Calvo JF, Ramírez-Díaz L. 2000. Recovery of germination from different osmotic conditions by four halophytes from southeastern Spain. *Annals of Botany* **85**: 279–286.
- R Core Team. 2021. *R: A language and environment for statistical computing*. Vienna: The R Foundation for Statistical Computing. <https://www.R-project.org>.
- Raddi S, Mariotti B, Martini S, Pierguidi A. 2019. Salinity tolerance in *Fraxinus angustifolia* Vahl.: seed emergence in field and germination trials. *Forests* **10**: 940.
- Revell LJ. 2012. phytools: an R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution* **3**: 217–223. doi:10.1111/j.2041-210x.2011.00169.x.
- RStudio Team. 2022. *RStudio: integrated development environment for R*. Boston: RStudio, PBC. <http://www.rstudio.com>.
- Saeed S, Gul B, Khan M. 2011. Comparative effects of NaCl and sea salt on seed germination of *Arthrocnemum indicum*. *Pakistan Journal of Botany* **43**: 1091–1103.
- Santo A, Mattana E, Hugot L, Spinosi P, Bacchetta G. 2014. Seed germination and survival of the endangered psammophilous *Rouya polygama* (Apiaceae) in different light, temperature and NaCl conditions. *Seed Science Research* **24**: 331–339. doi:10.1017/s0960258514000282.
- Santo A, Mattana E, Frigau L, Marzo Pastor A, Picher Morello MC, Bacchetta G. 2017. Effects of NaCl stress on seed germination and seedling development of *Brassica insularis* Moris (Brassicaceae). *Plant Biology* **19**: 368–376. doi:10.1111/plb.12539.
- Seneviratne SI, Nicholls N, Easterling D, et al. 2012. Changes in climate extremes and their impacts on the natural physical environment. In: Field CB, Barros V, Stocker TF, et al. eds. *Managing the risks of extreme events and disasters to advance climate change adaptation A Special Report of Working Groups I and II of the Intergovernmental Panel on Climate Change (IPCC)*. Cambridge: Cambridge University Press, 109–230.
- Suarez N. 2011. Effects of short- and long-term salinity on leaf water relations, gas exchange, and growth in *Ipomoea pes-caprae*. *Flora* **206**: 267–275. doi:10.1016/j.flora.2010.05.006.
- UNEP. 2006. *Marine and coastal ecosystems and human well-being: a synthesis report based on the findings of the Millennium Ecosystem Assessment*. Nairobi, Kenya: United Nations Environment Programme.

- USFWS. 2023. *U.S. Fish and Wildlife Service Endangered Species*. <https://www.fws.gov/program/endangered-species/species>. (8 March 2023, date last accessed).
- Viechtbauer W. 2014. Conducting meta-analyses in R with the metafor package. *Journal of Statistical Software* **36**: 1–48.
- Warshauer FR, Jacobi JD, Price J. 2009. *Native coastal flora and plant communities in Hawai'i: Their composition, distribution, and status*. Hilo, HI: Hawaiian Cooperative Studies Unit Technical Report HCSU-014. University of Hawai'i at Hilo.
- Wickham H. 2016. *ggplot2: elegant graphics for data analysis*. New York: Springer.
- Wolkis D, Baskin CC, Baskin JM, Rønsted N. 2022. Seed dormancy and germination of the endangered exceptional Hawaiian lobelioid *Brighamia rockii*. *Applications in Plant Sciences* **10**: e11492. doi:10.1002/aps3.11492.
- Woodell SRJ. 1985. Salinity and seed germination patterns in coastal plants. *Vegetatio* **61**: 223–229. doi:10.1007/bf00039828.
- Yousefi S, Kartoolinejad D, Bahmani M, Naghdi R. 2017. Salinity tolerance of *Dodonaea viscosa* L. inoculated with plant growth-promoting rhizobacteria: assessed based on seed germination and seedling growth characteristics. *Folia Oecologica* **44**: 20–27. doi:10.1515/foecol-2017-0003.
- Zhu JK. 2000. Genetic analysis of plant salt tolerance using *Arabidopsis*. *Plant Physiology* **124**: 941–948. doi:10.1104/pp.124.3.941.
- Zhu JK. 2001. Plant salt tolerance. *Trends in Plant Science* **6**: 66–71. doi:10.1016/s1360-1385(00)01838-0.
- Zunzunegui M, Esquivias MP, Fernández-González P, Valera-Burgos J, Díaz Barradas MC, Gallego-Fernández JB. 2017. Morphophysiological response of *Retama monosperma* to extreme salinity levels. *Ecophysiology* **10**: e1871. doi:10.1002/eco.1871.

