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Phenotypic Variation Among Invasive *Phragmites australis* Populations Does Not Influence Salinity Tolerance

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Abstract Phenotypic variation within species can have community- and ecosystem-level effects. Such variation may be particularly important in ecosystem engineers, including many invasive species, because of the strong influence of these species on their surrounding communities and environment. We combined field surveys and glasshouse experiments to investigate phenotypic variation within the invasive common reed, *Phragmites australis*, among four estuarine source sites along the east coast of North America. Field surveys revealed variation in *P. australis* height and stem density among source sites. In a glasshouse environment, percent germination of *P. australis* seeds also varied across source sites. To test the degree to which phenotypic variation in *P. australis* reflected genetic or environmental differences, we conducted a glasshouse common garden experiment assessing the performance of *P. australis* seedlings from the four source sites across a salinity gradient. Populations maintained differences in morphology and growth in a common glasshouse environment, indicating a genetic component to the observed phenotypic variation. Despite this variation, experimentally increased porewater salinity consistently reduced *P. australis* stem density, height, and biomass. Differences in these morphological metrics are important because they are correlated with the impacts of invasive *P. australis* on the ecological communities it invades. Our results indicate that both colonization and spread of invasive *P. australis* will be dependent on the environmental and genetic context. Additional research on intraspecific variation in invasive species, particularly ecosystem engineers, will improve assessments of invasion impacts and guide management decisions in estuarine ecosystems.

Keywords Common garden · Ecosystem engineer · Germination · Intraspecific variation · Invasive species · Salt marsh

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Introduction

Many invasive species, particularly plants, act as ecosystem engineers in their invaded range, causing changes in hydrological regimes, nutrient cycling, and primary production (e.g., *Spartina alterniflora*, Neira et al. 2005; *Phragmites australis*, Hazelton et al. 2014). Phenotypic variation within ecosystem engineers, including those that are invasive, can influence the effects of these species on community properties and ecosystem function (Whitham et al. 2003). In addition, phenotypic variation in fitness (e.g., seed production, seed viability, and/or above- and below-ground biomass) also affects the impacts of invasive ecosystem engineers by mediating their spread within and across habitats (Mateos-Naranjo and Redondo-Gomez 2015; Grewell et al. 2016). Given that phenotypic variation, particularly in ecosystem engineers, can have strong effects on population, community, and ecosystem processes, the role of phenotypic variation in the spread and impacts of invasive species deserves greater attention

(Hastings et al. 2005; Hughes et al. 2008; Williams and Grosholz 2008; Bolnick et al. 2011; Hazelton et al. 2014).

Coastal habitats are especially vulnerable to invasions (Zedler and Kercher 2004): the rate of reported marine and estuarine invasions in the USA has increased exponentially over the past 200 years (Ruiz et al. 2000), with the establishment of approximately 400 invasive species in these habitats (Ruiz et al. 1997). In addition, over half of marine invasive species are classified as ecosystem engineers (Katsanevakis et al. 2014). For example, invasive clams have dramatically altered community structure and nutrient cycling in San Francisco Bay (Chauvaud et al. 2003; Greene et al. 2011). Similarly, the establishment of the invasive cordgrass (*S. alterniflora*) in the mudflats of San Francisco Bay has modified the hydrodynamic regime, triggering physical, chemical, and biological changes in this ecosystem (Neira et al. 2006). Predicting the performance and impacts of invasive species is complicated by the fact that effects within a particular estuary or coastal region may not be reliably extrapolated to other systems (Ruiz et al. 1999; Grosholz 2001). For instance, invasion of the submerged plant, *Hydrilla verticillata*, in Florida waterways negatively affected fish populations (Langeland 1996), but invasion of *H. verticillata* in the Chesapeake region increased fish populations (Killgore et al. 1989; Serafy et al. 1994). The opposing impacts of invasive *H. verticillata* on fish populations are correlated with phenotypic differences in *H. verticillata* between regions (Ruiz et al. 1999). Thus, while invasive species can have profound effects on estuarine ecosystems, the magnitude and direction of their effects can vary as a result of phenotypic variation among locations, suggesting that consideration of the effects of phenotypic variation within invasive ecosystem engineers may provide valuable information for the management of estuarine invasions (Ruiz et al. 1999; Grosholz 2002; Hazelton et al. 2014).

Many estuaries along the eastern coast of North America are currently dominated by an invasive lineage (haplotype M) of the wetland grass *P. australis* (Cav.) Trin. ex Steudel, hereafter referred to as invasive *P. australis* (Saltonstall 2002). Invasive *P. australis* first appeared in herbarium records in the late 1800s and has since largely replaced the multiple native *P. australis* haplotypes, as well as other native marsh plant species (e.g., *S. alterniflora*), in these estuarine communities (Chambers et al. 1999; Meyerson et al. 2000; Saltonstall 2002). Invasive *P. australis* has also invaded freshwater habitats, roadside ditches, and other disturbed areas (Marks et al. 1994; Saltonstall 2002). Invasive *P. australis* alters estuarine ecosystems via impacts on nutrient cycling and marsh elevation, and it can negatively affect associated fauna including birds and fish (Keller 2000; Talley and Levin 2001; Burdick and Konisky 2003; Able et al. 2003; Minchinton et al. 2006). Invasive *P. australis* is generally viewed as a nuisance, and has become the focus of labor-intensive and expensive management or eradication efforts (Martin and Blossey 2013; Hazelton et al. 2014).

There is increasing evidence that the invasion potential and ecological consequences of invasive *P. australis* vary across populations. For instance, variation in seed viability between *P. australis* patches both within and among estuaries provides strong evidence that the invasive potential of *P. australis* populations varies (Kettenring and Whigham 2009; Kettenring et al. 2010). In addition, invasive populations differ in vegetative morphology, physiology, plant-herbivore interactions, nutrient use efficiency, and many other variables along the east coast of North America, and these differences can have important effects on associated plant and animal communities (King et al. 2007; Cronin et al. 2015; Hughes et al. 2016; Mozder et al. 2016). Variation in vegetative morphology across a latitudinal gradient was also observed in native *P. australis* from European and American populations (Clevering et al. 2001; Saltonstall and Court Stevenson 2007).

Phenotypic differences among populations of invasive *P. australis* may be driven by local environmental variation (i.e., phenotypic plasticity), genetic variation (e.g., local adaptation), or a combination of environmental and genetic factors. For instance, shorter *P. australis* haplotypes are generally more salt tolerant (Achenbach et al. 2013; Achenbach and Brix 2013) suggesting that phenotypic variation in height may be an evolutionary response to salinity stress. However, differences in height among populations may also be the result of plastic responses to the environment, including salinity. Increases in salinity above 10 ppt typically trigger plastic phenotypic responses in invasive *P. australis* populations, including decreased height, biomass production, stem production, and relative growth rate (Mauchamp and Mésleard 2001; Vasquez et al. 2005; Howard 2010; Achenbach et al. 2013). Understanding which mechanisms contribute to phenotypic variability in invasive *P. australis* can advance our understanding of invasion dynamics. For example, investigating the causes of variability among invader populations will help determine how frequently contemporary evolution occurs following population bottlenecks and, when it does, the degree to which evolution influences the subsequent spread of a species (Weber and Schmid 1998; Stockwell et al. 2003; Sax et al. 2007). Understanding the relative contribution of genetic versus environmental factors to invasive *P. australis* traits will also help improve management strategies. Evolution can work against traditional approaches to control weedy species, such as the mass application of herbicides, because these control measures exert strong selection on the target species and thus result in the evolution of resistance (Barrett 2000). We chose salinity as our focal environmental factor because it strongly affects many *P. australis* traits associated with the effects of *P. australis* on community properties and ecosystem function (Achenbach et al. 2013) and varies among estuaries along the east coast of North America that support *P. australis* (Hughes

et al. 2016). Determining whether or not invasive *P. australis* populations exhibit local adaptation to stressors like salinity will help managers identify how best to implement such stressors to obtain control and eradication objectives (Stockwell et al. 2003; Hazelton et al. 2014).

We investigated the causes and consequences of phenotypic variation among invasive *P. australis* populations with a combination of observational and experimental data. (1) We conducted a *field phenotypic survey* to assess our hypothesis that stem and panicle morphology vary among invasive *P. australis* populations from four source sites along the eastern coast of North America. (2) Then, we performed a *germination assay* to investigate our hypothesis that percent germination of invasive *P. australis* seeds varies among the four source sites and, because panicle and seed morphology may affect percent germination of seeds (Martincic et al. 1997; Friedman and Barrett 2009), we investigated whether invasive *P. australis* percent germination is correlated with invasive *P. australis* panicle morphology and seed condition. (3) Finally, we conducted a *glasshouse salinity experiment* to investigate our hypothesis that variation in invasive *P. australis* morphology and salinity tolerance among the four source sites is due to genetic differences among *P. australis* populations.

Methods

Field Phenotypic Survey

In September–October 2014, we conducted a field survey of invasive *P. australis* from populations across four source sites in Massachusetts (MA; 42° 44.473′ N, 70° 51.043′ W), New Jersey (NJ; 39° 32.887′ N, 74° 27.727′ W), Delaware (DE; 39° 5.294′ N, 75° 26.274′ W), and Virginia (VA; 37° 13.160′ N, 76° 24.758′ W) (Hughes et al. 2016). Source sites were chosen to represent spatially distinct invasive *P. australis* populations distributed across the range of invasion along the Eastern Coast of the USA. Via restriction fragment length polymorphism genetic analyses of two non-coding chloroplast DNA regions, we confirmed that all sampled *P. australis* were of the invasive European lineage (Saltonstall 2003; Hughes et al. 2016). *P. australis* populations in the Eastern USA estuaries separated by greater than 1 km are often genetically distinct (McCormick et al. 2010). The distances between source sites in our study were on the order of tens to hundreds of kilometers; thus, it is probable that *P. australis* from each source site represent genetically distinct populations.

At each source site, we haphazardly placed 12–18 0.25 m^2 quadrats (separated by ≥ 5 m) in interior (> 5 m from the patch edge) locations within stands of *P. australis*. In each quadrat, we enumerated vegetative (non-flowering) and flowering *P. australis* stems and recorded the height of the tallest vegetative stem. In addition, we harvested up to three panicles from separate *P. australis* stems in each quadrat and stored them at 4 °C until processing. We measured length (cm), width at midpoint (cm), and weight (g) of each panicle and enumerated spikelets on each panicle. For one panicle per quadrat, we haphazardly chose three spikelets for seed counts.

In each quadrat, we also measured the salinity of porewater collected from a depth of 10 cm using a handheld refractometer (Atago 2491 Master-S/Mill∞) following standard methods. To complement these point estimates of porewater salinity, we obtained open water salinity measurements for NJ, DE, and VA source sites over 5 years (2010–2014) from the National Estuarine Research Reserve System's (NERRS) long-term water quality monitoring sites (http://cdmo.baruch.sc.edu/). For the MA source site, we used 4 years (2010–2013) of salinity data collected at the Plum Island Ecosystems Long-Term Ecological Research (PIE-LTER) station's experimental research sites (https://portal.lternet.edu.nis/home.jsp). All open water salinity sites were located < 2 km from our source sites. Despite the extensive temporal coverage of open water salinity measurements and the spatial coupling of open water measurements at each site with the focal *P. australis* stands, discrepancies between open water salinity and site salinity may still exist due to unaccounted for plot level factors such as tidal currents and terrestrial runoff.

Germination Assay

Immediately after processing panicles collected in the field survey, we haphazardly chose one spikelet from each panicle to prepare for a subsequent seed germination assay. We wrapped each spikelet in filter paper and buried these spikelets in moist soil at 4 °C for 5 weeks to break seed dormancy (Kettenring and Whigham 2009).

On December 15, 2014, we planted up to 100 seeds from each spikelet on the surface of wet sand in 10-cm-diameter petri dishes (Saltonstall and Court Stevenson 2007; Kettenring and Whigham 2009). Throughout the germination period, we kept the seeds under ambient light conditions in a glasshouse at the Northeastern University Marine Science Center, Nahant, MA (MSC glasshouse). We maintained temperature above 4 °C using an electric heating system, and we added distilled water (0 ppt) as necessary to insure the sand remained moist. Upon the start of germination in January 2015, we transplanted groups of up to five seedlings per source quadrat into 7 × 7 × 6.3-cm pots containing commercial potting soil, and we watered pots daily for 1.5 h at a rate of 0.3 L freshwater pot^{-1} h^{-1} from overhead sprinklers in the glasshouse. Germination did not occur after mid-February, so we ended the germination trial in March. In early May, we transplanted individual *P. australis* seedlings into separate pots (9 × 9 × 24 cm) containing a 1:1 ratio of commercial potting soil and sand. Over the following 3-week acclimation period,

we watered pots daily for 1.5 h at a rate of 0.45 L freshwater pot^{-1} h^{-1} with freshwater from overhead sprinklers in the glasshouse.

Glasshouse Salinity Experiment

We conducted a salinity experiment in the MSC glasshouse. We evaluated *P. australis* growth traits (maximum stem height, total stem density, above-ground biomass, total below-ground biomass, root biomass, and rhizome biomass) using a split plot design with comparisons between two factors: (1) source site (split plot factor): MA, NJ, DE, and VA and (2) porewater salinity (whole plot factor): 0, 12, 17, and 22 ppt. On May 25, we randomly assigned pots containing individual *P. australis* seedlings from each of the four source sites to 16 40 × 28 × 17-cm bins and randomly assigned each bin to one of four target porewater salinity treatments (two plants from each source site per bin and four bins per salinity treatment, for a total of 128 individual plants) (Fig. ESM 1). We mixed bins randomly across two water tables (i.e., blocks), and we randomly re-assigned bin locations within water tables on a monthly basis to avoid position specific effects (i.e., shading) (Fig. ESM 1).

We manipulated porewater salinity within the pots by varying the frequency of 8-h saltwater (~ 30 ppt from the MSC glasshouse seawater system) and freshwater (~ 0 ppt from the MSC glasshouse irrigation system) soaks across bins (Table 1). To soak the pots, we drilled a 1.5-cm-diameter hole in the bottom of each bin and fitted these holes with a rubber stopper to allow regulation of the flow of water into and out of the bins. For saltwater soaks, we filled the water tables to 3 cm below the rim of each bin. Saltwater was delivered into the bottom of the bins via the hole in each bin. Immediately prior to saltwater soaks, we plugged bins receiving freshwater soaks with rubber stoppers and added freshwater to 3 cm below the rim of each bin via irrigation hoses running into the bottom of the bin. After 8 h, we drained the saltwater from the bins and water tables. Immediately after the saltwater completely drained (~ 5 min), we drained the bins receiving freshwater by removing the rubber stoppers. We were careful to position the bin drains so that no draining freshwater entered bins previously soaked in saltwater. We drained all bins via gravity. Except for the first 5 days of the experiment when we soaked the pots each day in order to reach the targeted soil salinities, we soaked plants one to three times per week. In addition to the soaks, we irrigated all plants twice a week with freshwater via the glasshouse irrigation system overhead sprinklers for 1.5 h at a rate of 0.45 L freshwater pot^{-1} h^{-1}. We only irrigated plants on days when they were not already receiving soaks. Otherwise, we maintained plants in dry conditions.

Table 1 Experimental salinity treatments from glasshouse common garden salinity experiment

Target salinity (ppt)	Mean actual salinity (ppt) [SE]	No. of freshwater soaks: no. of seawater soaks
0	1.13 [0.26]A	32:0
12	12.28 [1.22]B	20:12
17	16.98 [1.22]C	10:22
22	21.58 [1.45]D	2:30

Letters indicate significant groupings from pairwise Wilcox rank-sum tests ($\alpha \leq 0.05$)

We monitored source water and porewater salinity one to three times per week during soaks over the course of the experiment using a YSI 556 Handheld Multiparameter Instrument with a 556 DO/Temperature/Conductivity Field Cable. We measured porewater salinity in two randomly selected pots from each salinity treatment at a soil depth of 10 cm and modified the frequency of saltwater soaks as needed to maintain target porewater salinities over the course of the experiment. Throughout the experiment, plants were exposed to ambient light conditions and we maintained temperatures between 4 and 27 °C via evaporative cooling and electric heating.

At the start of the experiment, we measured the height of the tallest stem and enumerated live and dead stems for each plant. After 110 days, we again measured the height of the tallest stem and enumerated live and dead stems for each plant. In addition, we harvested the plants and measured above-ground biomass for each plant. For a subset of 83 plants containing at least one replicate per source site per bin, we measured below-ground biomass. We separated the below-ground biomass samples into roots and rhizomes and measured the biomass of these below-ground structures separately for each of the 83 plants sampled. We dried all above- and below-ground samples at 70 °C for 72 h before measuring biomass.

Statistical Analyses

Field Phenotypic Survey We used one-way analysis of variance (ANOVA) to examine differences in vegetative *P. australis* maximum stem height, point estimated porewater salinity, and long-term mean monthly salinity with source site as the categorical fixed effect. We used analysis of covariance (ANCOVA) to examine differences in flowering *P. australis* stems per 0.25 m^2 among source sites, with source site as a categorical fixed effect and *P. australis* stem density as a covariate in the model. We then used Tukey honest significant difference (HSD) post hoc tests to differentiate among source sites. Although analyses were conducted as ANCOVA, data are presented as percentages for ease of interpretation. We square root-transformed vegetative *P. australis* maximum stem height to meet the assumptions of ANOVA; for transformed data, we present geometric means and standard errors

in the figures. We were unable to correct for non-normality in vegetative *P. australis* stem density using transformations. Therefore, we used a non-parametric Kruskal-Wallis *H* test to examine differences in *P. australis* vegetative stem density among source sites, followed by Mann-Whitney *U* tests for pairwise comparisons.

For each quadrat, we calculated mean panicle length, width, and weight; number of spikelets per panicle; and number of seeds per spikelet. We square root-transformed panicle weight and spikelet number per panicle to meet the assumptions of ANOVA. All panicle traits were significantly correlated with one another (Fig. ESM 2, pairwise Pearson product-moment correlations, df = 50, $P < 0.05$); therefore, we used a multivariate analysis of variance (MANOVA) to examine differences in panicle morphology among source sites. In the case of a significant effect of source site in the MANOVA, we then used one-way ANOVA models followed by Tukey HSD post hoc tests to investigate the effects of source site on each panicle trait.

Germination Assay We used Pearson's χ^2 test to examine differences in percent germination among source sites. We used generalized linear mixed-effects models (GLM) to examine relationships between percent germination and panicle traits. A logistic link function was applied to account for percent germination being a proportional variable, and site was included as a random effect in the models. We tested for both linear and quadratic relationships in the GLM models. Quadratic models were included in order to capture potential non-linear effects of panicle traits on percent germination. We dropped quadratic relationships from the models if they were not significant. Because there is a high degree of correlation among panicle traits, we conducted a principal component analysis (PCA) of panicle traits prior to GLM analyses in order to reduce the dimensionality and covariance of the data (Gotelli and Ellison 2013). Prior to the PCA, we Z-score-transformed each panicle trait so that the axes of the PCA are not dominated by variables that have large units of measurement (Gotelli and Ellison 2013). We retained principal components for subsequent GLM analyses following Kaiser-Guttman and broken stick methods to identify informative principal components (Jackson 1993).

Glasshouse Salinity Experiment Due to unequal variances among porewater salinity treatments, we used a non-parametric Kruskal-Wallis *H* test to confirm differences in porewater salinity, followed by Mann-Whitney *U* tests for pairwise comparisons. We used two-way multivariate analysis of covariance (MANCOVA) to examine differences in above- and below-ground *P. australis* growth traits (stem height, stem number, and above-ground, below-ground, rhizome, and root biomass) between salinity treatments and source sites. We treated source site as a categorical fixed effect and salinity treatment as a continuous covariate in the MANCOVA models, and we nested source site within bin as a random effect to account for the split plot design. Specifically, salinity was analyzed as a whole plot factor and source site was analyzed as a split plot factor. We also included water table as a random effect in the models. In the case of significant independent or interactive effects of salinity treatment or source site in the MANCOVA models, we then used two-way ANCOVA models to examine the independent and interactive effects of salinity treatment and source site on individual *P. australis* growth traits. For salinity treatment analyses, we tested for both linear and quadratic relationships in the ANCOVA models. Quadratic models were included in order to capture potential non-linear effects of salinity common in organismal responses to stressors (e.g., thermal performance curves). We dropped quadratic relationships from the models if they were not significant. We used initial stem height and density as covariates in individual stem height and density ANCOVA models, respectively. We used Tukey HSD post hoc tests to differentiate *P. australis* growth traits among source sites.

We conducted all analyses using R version 3.3.2 (R Core Team 2016) in the RStudio platform version 1.0.136 (RStudio 2016). We used R packages car 2.1-4, corrgram 1.10, dplyr 0.5.0, ggplot2 2.2.1, lme4 1.1-12, lmerTest 2.0-33, lsmeans 2.25, multcompView 0.1-7, nlme 3.1-130, sciplot 1.1-0, tidyr 0.6.1, vegan 2.4-2, and zoo 1.7-14 compatible with R version 3.3.2 to analyze data and create figures. For all analyses, we considered results significant at $\alpha \leq 0.05$.

Results

Field Phenotypic Survey

P. australis vegetative stem height differed significantly across source sites (Fig. 1a, ANOVA, square root-transformed, $F_{3,55} = 2.92$, $P = 0.042$): average stem height in NJ was 28% greater than in MA, while *P. australis* heights in DE and VA were intermediate and not significantly different from other source sites (Fig. 1a). Porewater salinity (ppt) was also significantly different across source sites (Fig. 1b, ANOVA, $F_{3,46} = 22.63$, $P < 0.01$). Point-estimated porewater salinity followed the opposite pattern to *P. australis* vegetative stem height: porewater salinity in MA, VA, and DE was 83, 66, and 59% greater than in NJ, respectively (Fig. 1b). Long-term salinity was significantly different among source sites (Fig. 1b, ANOVA, $F_{3,188} = 120.54$, $P < 0.01$). Similar to point-estimated porewater salinity, long-term salinity in MA and VA was 97 and 37% greater than in NJ, but long-term salinity in DE was 21% less than in NJ (Fig. 1b, Table ESM 1). *P. australis* vegetative stems per 0.25 m^2 also

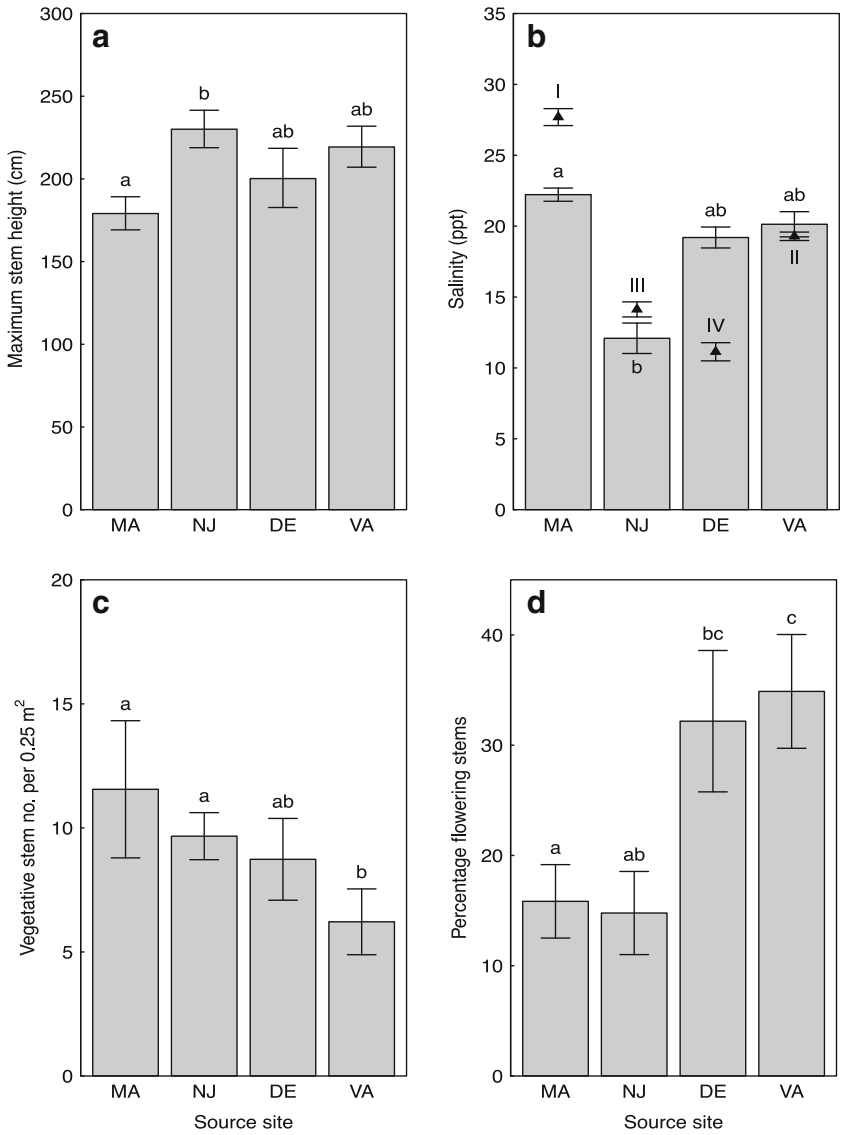

Fig. 1 Relationships of *P. australis* morphology and porewater salinity among source sites. Heights of bars (**a**–**d**) and data points (**b**) represent the mean; error bars represent ± 1 SE. Letters indicate significant groupings of bars, and roman numerals represent significant groupings of points from **a, b**, **d** Tukey HSD post hoc tests or **c** pairwise Mann-Whitney *U* tests (both $\alpha \leq 0.05$). Bars (**b**) represent mean porewater salinity at source sites measured at the time of sampling, and black triangles (**b**) represent mean long-term salinity at source sites

varied significantly among source sites (Fig. 1c, Kruskal-Wallis test, $\chi^2_3 = 7.91$, $P = 0.048$), with vegetative stem density in MA and NJ 87 and 57% higher than in VA (Fig. 1c). Stem density in DE was not significantly different from MA, NJ, or VA (Fig. 1c). *P. australis* flowering stem number also varied among source sites, after accounting for variation in vegetative stem density (Fig. 1d, ANCOVA, $F_{3,45} = 6.58$, $P < 0.01$). A greater percentage of *P. australis* stems in VA and DE were flowering than in MA (Fig. 1d). The percentage of flowering stems in VA was also greater than in NJ (Fig. 1d). There were no other significant differences in flowering stem percentage among all other pairwise site comparisons (Fig. 1d).

P. australis panicle morphology varied significantly among source sites (MANOVA, $F_{15,138} = 3.31$, $P < 0.01$), due to significant differences in panicle length (ANOVA, length $F_{3,48} = 7.98$, $P < 0.01$). The width, weight, number of spikelets per panicle, and number of seeds per spikelet did not vary significantly among source sites. Panicles collected from VA (25.19 cm ± 1.60 S.E.) were 68 and 43% longer than panicles collected from MA (15.00 cm ± 1.40 S.E.) and NJ (17.56 cm ± 2.04 S.E.). Panicles collected from DE (20.07 cm ± 1.54 S.E.) did not vary significantly in length from the other source sites.

Germination Assay

P. australis percent germination varied significantly across source sites (Fig. 2a, Pearson's χ^2 test, $\chi^2_3 = 175.36$, $P < 0.01$). The proportion of seeds from NJ and DE successfully germinating was approximately three times higher than seeds from MA and VA. The first principal component (PC1) of the panicle trait PCA explained 75% of the variance among panicle traits, and no other component explained more than

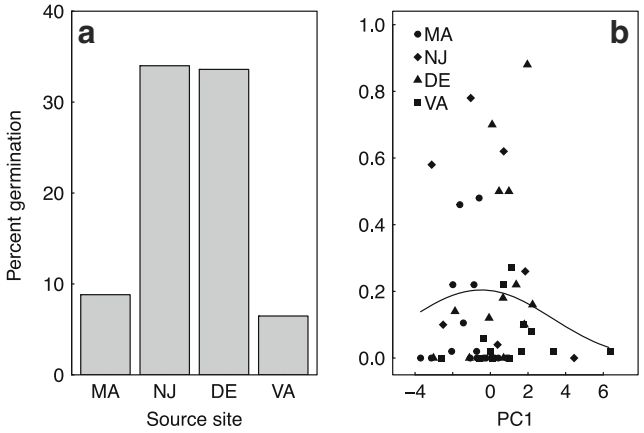

Fig. 2 Relationships between *P. australis* seed percent germination and **a** source site and **b** principal component 1 (PC1) from a principal component analysis of quadrat mean trait values: length, width, spikelet number, seed number per spikelet, and weight. **a** Heights of bars represent percent germination among source sites. **b** Points represent quadrat percent germination by PC1 values; solid lines represent significant relationships from GLMER

12% of the remaining variance among panicle traits (Table ESM 2). We only retained PC1 for subsequent analyses in accordance with Kaiser-Guttman and broken stick methods (Jackson 1993) (Fig. ESM 3, Table ESM 2). All the loadings along the PC1 axis are positive, and all are of approximately the same magnitude (Table ESM 2). Thus, PC1 seems to be a good measure of panicle size—large, heavy panicles with many spikelets and many seeds per spikelet will have larger PC1 values. There was a positive quadratic relationship between percent germination and PC1, and percent germination peaked at intermediate PC1 values (GLMER, $Z = -4.05$, $P < 0.01$) (Fig. 2b).

Glasshouse Salinity Experiment

Prior to the experiment, there was no relationship between porewater salinity treatment and the number of stems per *P. australis* seedling, nor did stem number differ among source sites (ANCOVA, salinity $F_{1,13.99} < 0.01$, $P = 0.97$; source site $F_{3,39.18} = 1.10$, $P = 0.36$). Similarly, there was no relationship between porewater salinity and initial maximum height of *P. australis* seedlings, nor did initial maximum height differ among source sites (ANCOVA, salinity $F_{1,13.14} = 0.03$, $P = 0.87$; source site $F_{3,99.05} = 1.01$, $P = 0.39$).

During the experiment, porewater salinity differed significantly among treatments (Kruskal-Wallis test, $\chi^2_3 = 56.27$, $P < 0.01$), with all treatments significantly different from each other (Table 1). There were significant independent effects of salinity treatment and source site on above-ground *P. australis* responses, but no salinity * source site interaction (MANCOVA, salinity $F_{1,14} = 5.45$, $P = 0.04$; source site $F_{3,45} = 5.40$, $P < 0.01$). When we examined responses individually, both salinity treatment and source site had independent effects on *P. australis* stem number, stem height, and above-ground biomass (Table ESM 3). *P. australis* from MA produced 33% more stems than *P. australis* from NJ, DE, and VA, which did not differ from one another (Fig. 3a). However, the maximum stem height and above-ground biomass of *P. australis* from MA were 15–20 and 20–30% less, respectively, than the maximum stem height and above-ground biomass of *P. australis* from NJ, DE, and VA, which again did not differ among one another (Fig. 3b, c). There was also a significant negative linear relationship between porewater salinity and *P. australis* stem production (Fig. 3d). In contrast, there were negative quadratic relationships between porewater salinity and *P. australis* maximum height and above-ground biomass (Fig. 3e, f).

There were also significant effects of salinity treatment and source site on below-ground *P. australis* responses, but no interactive effects of salinity * source site (MANCOVA, salinity $F_{1,14} = 126.07$, $P < 0.01$; source site $F_{3,42} = 5.05$, $P < 0.01$). In individual ANCOVAS, salinity treatment and source site had significant independent effects on *P. australis* total below-ground biomass and root biomass (Table ESM 3). The below-ground biomass of *P. australis* from MA was 21 and 23% less than *P. australis* from DE and VA, respectively, and below-ground biomass did not differ significantly between *P. australis* from NJ and *P. australis* from the other three source sites (Fig. 4a). Similarly, root biomass of *P. australis* from MA was 25 and 34% less than *P. australis* from NJ and VA, respectively, and root biomass did not differ significantly between *P. australis* from DE and *P. australis* from the other three source sites (Fig. 4b). There were negative quadratic relationships between porewater salinity and *P. australis* below-ground biomass and root biomass (Fig. 4d, e). Salinity treatment, but not source site, had a significant effect on *P. australis* rhizome biomass (Table ESM 3): there was a significant negative linear relationship between porewater salinity and rhizome biomass (Fig. 4f).

Discussion

Phenotypic differences among populations of invasive species, resulting from both phenotypic plasticity and genetic variation, can influence their distribution and effects within and among ecosystems (Richards et al. 2006; Fortune et al. 2008; Grosholz 2002; Hughes et al. 2016). In the field, we found that invasive *P. australis* vegetative morphology and the number of *P. australis* reproductive stems differed across four estuarine source sites along the mid-Atlantic and northeastern coast of the USA, yet this variation did not always follow expected correlations with porewater salinity at those same source sites. For instance, MA had higher porewater salinity than NJ, but the two source sites had equivalent stem density, suggesting that *P. australis* at our MA source sites

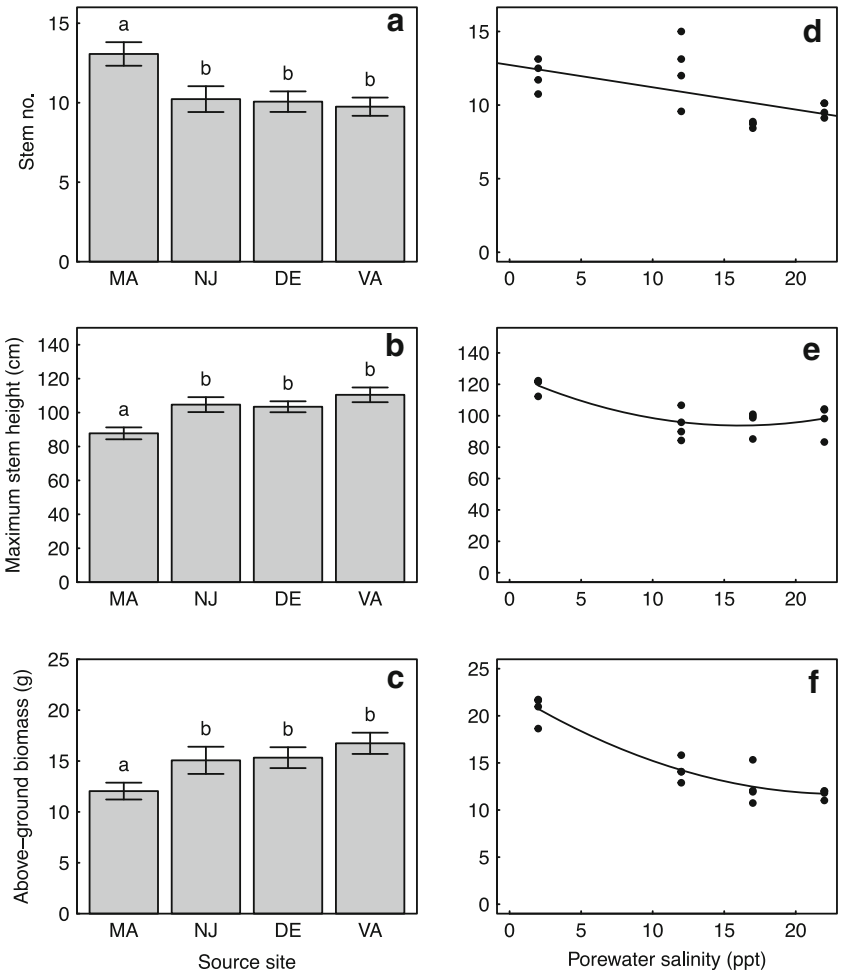

Fig. 3 Relationships of *P. australis* above-ground traits to source site and porewater salinity from glasshouse common garden salinity experiment. **a**–**c** Heights of bars represent source site means; error bars represent ± 1 SE. Letters indicate significant groupings from Tukey HSD post hoc tests ($\alpha \leq 0.05$). **d**–**f** Filled circles represent bin means for each salinity treatment; solid lines represent significant relationships from ANCOVA

may be adapted to higher salinity. Although populations maintained differences in morphology and growth in a common glasshouse environment, suggesting these differences were due in part to evolutionary processes such as local adaptation, experimentally increased porewater salinity consistently reduced stem number, height, and biomass of invasive *P. australis* seedlings germinated from all source sites. Thus, our common garden experiment did not support our hypothesis that invasive *P. australis* from MA was adapted to local salinity conditions, but rather confirmed prior findings that invasive *P. australis* populations exhibit similar phenotypic plasticity in response to salt stress (i.e., are equally susceptible) regardless of geographic origin or genetic background (Lissner and Schierup 1997; Vasquez et al. 2005; Achenbach et al. 2013).

Spatial and/or temporal refuges could account for the discrepancy in the relationship between porewater salinity and *P. australis* morphology that we observed in the field. For instance, our field sampling did not account for variation in porewater salinity over time or across soil depths, both potentially important salinity refuges for *P. australis* (Lissner and Schierup 1997; Burdick et al. 2001). While long-term water salinity data from the four source sites in our study suggests different sites experienced different salinity regimes, there was also substantial variation in these data. Seasonal variation in climate or monthly tidal cycles could result in relatively short-lived high-salinity events more tolerable for *P. australis* than sustained high salinity (Burdick et al. 2001; Mauchamp and Mésleard 2001). In addition, physiological integration of *P. australis* across expansive rhizome networks could also provide a spatial refuge, leading to disparities between *P. australis* morphology and local environmental conditions (Hara et al. 1993; Amsberry et al. 2000).

Morphological differences between populations in our glasshouse experiment may also reflect adaptations to other environmental factors that differ among our source sites. For instance, vegetative *P. australis* from the MA source site, the northern-most population in our study, produced many short stems in both the field and the common garden environment. A similar reduction in stem height and increase in stem number with increasing latitude of origin was observed in invasive *P. australis* grown from seeds collected from European populations (Clevering et al. 2001), as well as in a glasshouse common garden experiment involving native populations of

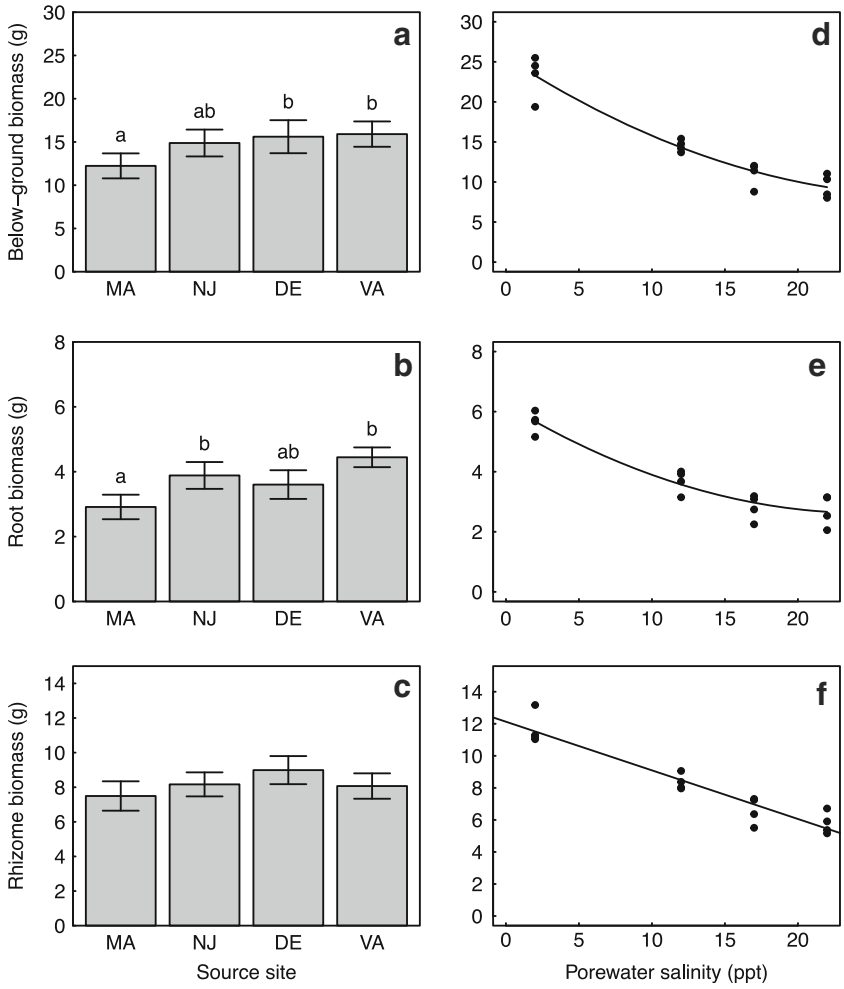

Fig. 4 Relationships of *P. australis* below-ground traits to source site and porewater salinity from glasshouse common garden salinity experiment. **a**–**c** Heights of bars represent source site means; error bars represent ± 1 SE. Letters indicate significant groupings from Tukey HSD post hoc tests ($\alpha \leq 0.05$). **d**–**f** Filled circles represent bin means for each salinity treatment; solid lines represent significant relationships from ANCOVA

P. australis collected from eastern USA and Canada (Saltonstall and Court Stevenson 2007). Both density-independent selection by frost and density-dependent selection by competition for light have been posited as possible explanations for such trends in other wetland reeds (McNaughton 1966; McNaughton 1975). For instance, taller stems may be more susceptible to damage from the severe winter conditions of northern latitudes, resulting in a greater proportion of shorter stems in these populations. However, increasing competition for light with increasing length of the growing season is thought to be the main driver of morphological differences among native *P. australis* populations in Europe (De Kroon and Kalliola 1995; Clevering et al. 2001). We cannot distinguish among these explanations in our study since the MA population, which had the most numerous yet shortest stems, experiences the most severe winters and the shortest growing season. Furthermore, our study does not capture the full variation in phenotype across invasive *P. australis* populations because of the relatively small number of populations sampled. For example, field surveys of invasive *P. australis* in South Carolina show that these invasive *P. australis* produce taller stems than any of the invasive *P. australis* sampled in this study (Hughes et al. 2016). Experiments that directly test the fitness benefits of morphological differences between invasive *P. australis* populations across both environmental and latitudinal gradients are needed to further understand the causes and consequences of these patterns (e.g., Grosholz 2001).

We found that source site identity significantly influenced seed germination rates in the absence of salinity stress, similar to prior findings for *P. australis* populations farther south on the Atlantic coast (Kettenring et al. 2010). Although panicle length varied among source sites, seedling germination rates peaked at intermediate panicle trait values, suggesting that greater reproductive investment in panicles is not necessarily an indicator of seed quality. Further studies that investigate the links between *P. australis* panicle morphology and the aerodynamics of pollen release and capture have the potential to elucidate whether variation in panicle morphology among source sites may be adaptive in *P. australis* (Friedman and Barrett 2009). Unfortunately, an assessment of other known correlates with *P. australis* percent germination, intraspecific genetic diversity, and nutrient availability at the source sites was beyond the scope of our study (e.g., Kettenring and

Whigham 2009; Kettenring et al. 2010). In addition, sexual reproduction of *P. australis* is increasingly thought to play an important role in the spread of invasive *P. australis* within and among estuaries along the eastern coast of North America, and thus, measurement of germination rates may offer a means of gauging the expansion potential of *P. australis* populations (McCormick et al. 2010). However, differences in the stages of flower or seed development among source sites at the time of collection may also contribute to patterns of percent germination; the four populations in our study ranged across a latitudinal gradient and thus experienced different numbers of growing degree days (GDD) (Spencer and Ksander 2006). The number of GDDs often affects the development and flowering of plants, including other wetland species: northern plants develop faster and flower earlier than their southern counterparts (Bastlová et al. 2004). Percent germination in our study did not appear to follow a pattern that would suggest a relationship with source site latitude; however, this observation is not sufficient to exclude the possibility that *P. australis* flowering phenology affected our results.

Effective prioritization of management actions requires accurate assessments of invasion impacts, yet these impacts can be highly context dependent across time and space, even for the same invasive species (Grosholz 2002; Parker et al. 1999; Strayer et al. 2006; Ricciardi et al. 2013). A broad range of ecological and evolutionary processes can contribute to this variation, but invader population identity has received relatively little attention to date (Ricciardi et al. 2013; Tepolt 2015), despite increasing evidence that trait variation within and among populations can have substantial ecological effects (Grosholz 2001; Hughes et al. 2008; Bolnick et al. 2011). In estuarine habitats dominated by invasive *P. australis*, invader morphology (e.g., stem density) is correlated with species richness of associated plants and invertebrates (Hughes et al. 2016), and invasive *P. australis* nutrition and defense traits influence the strength of plant-herbivore interactions (Cronin et al. 2015). Thus, trait variation among populations may influence the impact of *P. australis* invasion. In addition, studies comparing *P. australis* lineages have identified traits both linked to the spread of invasive *P. australis* in North America (Mozder and Zieman 2010; Mozder and Megonigal 2012) and indicative of changes in the distribution of *P. australis* lineages under future environmental conditions (Mozder et al. 2016). Quantifying variation in these traits among populations of invasive *P. australis* is a natural starting point for efforts seeking to identify which traits best predict the spread and impact of individual populations of invasive *P. australis*. Further research on intraspecific variation in invasive species, particularly ecosystem engineers, will improve assessments of invasion impact and guide management decisions in estuarine ecosystems (Mateos-Naranjo and Redondo-Gomez 2015; Grewell et al. 2016).

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References

Able, Kenneth, Stacy Hagan, and Steven Brown. 2003. Mechanisms of marsh habitat alteration due to *Phragmites*: response of young-of-the-year mummichog (*Fundulus heteroclitus*) to treatment for *Phragmites*. *Estuaries* 26: 484–494.

Achenbach, Luciana, and Hans Brix. 2013. Can differences in salinity tolerance explain the distribution of four genetically distinct lineages of *Phragmites australis* in the Mississippi River Delta? *Hydrobiologia* 737: 5–23.

Achenbach, Luciana, Franziska Eller, Loc Xuan Nguyen, and Hans Brix. 2013. Differences in salinity tolerance of genetically distinct *Phragmites australis* clones. *AoB Plants* 5: plt019. https://doi.org/10.1093/aobpla/plt019.

Amsberry, Lindsay, Michael A. Baker, Patrick J. Ewanchuk, and Mark D. Bertness. 2000. Clonal integration and the expansion of *Phragmites australis*. *Ecological Applications* 10: 1110–1118.

Barrett, Spencer C.H. 2000. Microevolutionary influences of global changes on plant invasions. In *Invasive Species in a Changing World*, ed. Harold A. Mooney and Richard J. Hobbs, 115–139. Washington, DC: Island Press.

Bastlová, Daša, Hana Čížková, Marek Bastl, and Jan Květ. 2004. Growth of *Lythrum salicaria* and *Phragmites australis* plants originating from a wide geographical area: response to nutrient and water supply. *Global Ecology and Biogeography* 13: 259–271.

Bolnick, Daniel I., Priyanga Amarasekare, Márcio S. Araújo, Reinhard Bürger, Jonathan M. Levine, Mark Novak, Volker H.W. Rudolf, Sebastian J. Schreiber, Mark C. Urban, and David A. Vasseur. 2011. Why intraspecific trait variation matters in ecology. *Trends in Ecology and Evolution* 26: 183–192.

Burdick, David M., and Raymond Konisky. 2003. Determinants of expansion for *Phragmites australis*, common reed, in natural and impacted coastal marshes. *Estuaries* 26: 407–416.

Burdick, David M., Robert Buchsbaum, and Eric Holt. 2001. Variation in soil salinity associated with expansion of *Phragmites australis* in salt marshes. *Environmental and Experimental Botany* 46: 247–261.

Chambers, Randolph M., Laura A. Meyerson, and Kristin Saltonstall. 1999. Expansion of *Phragmites australis* into tidal wetlands of North America. *Aquatic Botany* 64: 261–273.

Chauvaud, Laurent, Janet K. Thompson, James E. Cloern, and Gérard Thouzeau. 2003. Clams as CO_2 generators: the *Potamocorbula amurensis* example in San Francisco Bay. *Limnology and Oceanography* 48: 2086–2092.

Clevering, Olga A., Hans Brix, and Jaroslava Lukavská. 2001. Geographic variation in growth responses in *Phragmites australis*. *Aquatic Botany* 69: 89–108.

Cronin, James T., Ganesh P. Bhattarai, Warwick J. Allen, and Laura A. Meyerson. 2015. Biogeography of a plant invasion: plant-herbivore interactions. *Ecology* 96: 1115–1127.

De Kroon, Hans, and Risto Kalliola. 1995. Shoot dynamics of the giant grass *Gynerium sagittatum* in Peruvian Amazon floodplains, a clonal plant that does show self-thinning. *Oecologia* 101: 124–131.

Fortune, P.M., K. Schierenbeck, D. Ayres, A. Bortolus, O. Catrice, S. Brown, and M.L. Ainouche. 2008. The enigmatic invasive

Spartina densiflora: a history of hybridizations in a polyploidy context. *Molecular Ecology* 17: 4304–4316.

Friedman, Jannice, and Spencer C.H. Barrett. 2009. Wind of change, new insights on the ecology and evolution of pollination and mating in wind-pollinated plants. *Annals of Botany* 103: 1515–1527.

Gotelli, Nicholas J., and Aaron M. Ellison. 2013. *A primer of ecological statistics*. 2nd ed. Sunderland: Sinauer Associates Inc..

Greene, Valerie E., Lindsay J. Sullivan, Janet K. Thompson, and Wim J. Kimmerer. 2011. Grazing impacts of the invasive clam *Corbula amurensis* on the microplankton assemblage of the northern San Francisco Estuary. *Marine Ecology Progress Series* 431: 183–193.

Grewell, Brenda, Jesús Castillo, Meghan S. Thomason, and Rebecca Drenovsky. 2016. Phenotypic plasticity and population differentiation in response to salinity in the invasive cordgrass *Spartina densiflora*. *Biological Invasions* 18: 2175–2187.

Grosholz, Edwin D. 2001. Small spatial-scale differentiation among populations of an introduced colonial invertebrate. *Oecologia* 129: 58–64.

Grosholz, Edwin D. 2002. Ecological and evolutionary consequences of coastal invasions. *Trends in Ecology and Evolution* 17: 22–27.

Hara, T., J. van Der Toorn, and J.H. Mook. 1993. Growth dynamics and size structure of shoots of *Phragmites australis*, a clonal plant. *Journal of Ecology* 81: 47–60.

Hastings, Alan, Kim Cuddington, Kendi F. Davies, Christopher J. Dugaw, Sarah Elmendorf, Amy Freestone, Susan Harrison, Matthew Holland, John Lambrinos, Urmilla Malvadkar, Brett A. Melbourne, Kara Moore, Caz Taylor, and Diane Thomson. 2005. The spatial spread of invasions: new developments in theory and evidence. *Ecology Letters* 8: 91–101.

Hazelton, Eric L.G., Thomas J. Mozdzer, David M. Burdick, Karin M. Kettenring, and Dennis F. Whigham. 2014. *Phragmites australis* management in the United States: 40 years of methods and outcomes. *AoB Plants* 6: plu001. https://doi.org/10.1093/aobpla/plu001.

Howard, Rebecca J. 2010. Intraspecific Variation in growth of marsh macrophytes in response to salinity and soil type: implications for wetland restoration. *Estuaries and Coasts* 33: 127–138.

Hughes, A. Randall, Brian D. Inouye, Marc T.J. Johnson, Nora Underwood, and Mark Velland. 2008. Ecological consequences of genetic diversity. *Ecology Letters* 11: 609–623.

Hughes, A. Randall, Forest R. Schenck, Jeanne Bloomberg, Torrance C. Hanley, Dongmei Feng, Tarik C. Gouhier, R. Edward Beighley, and David L. Kimbro. 2016. Biogeographic gradients in ecosystem processes of the invasive ecosystem engineer *Phragmites australis*. *Biological Invasions* 18: 2577–2595.

Jackson, Donald A. 1993. Stopping rules in principal components analysis: a comparison of heuristical and statistical approaches. *Ecology* 74: 2204–2214.

Katsanevakis, Stelios, Inger Wallentinus, Argyro Zenetos, Erkki Leppäkoski, Melih Ertan Çinar, Bayram Oztürk, Michal Grabowski, Daniel Golani, and Ana C. Cardoso. 2014. Impacts of invasive alien marine species on ecosystem services and biodiversity: a pan-European review. *Aquatic Invasions* 9: 391–423.

Keller, Barbara E.M. 2000. Plant diversity in *Lythrum*, *Phragmites* and *Typha* marhes, Massachusetts, USA. *Wetlands Ecology and Management* 8: 391–401.

Kettenring, Karin M., and Dennis F. Whigham. 2009. Seed viability and seed dormancy of non-native *Phragmites australis* in suburbanized and forested watersheds of the Chesapeake Bay, USA. *Aquatic Botany* 91: 199–204.

Kettenring, Karin M., Melissa K. McCormick, Heather M. Baron, and Dennis F. Whigham. 2010. *Phragmites australis* (common reed) invasion in the Rhode River subestuary of the Chesapeake Bay: disentangling the effects of foliar nutrients, genetic diversity, patch size, and seed viability. *Estuaries and Coasts* 33: 118–126.

Killgore, K. Jack, Raymond P. Morgan, and Nancy B. Rybicki. 1989. Distribution and abundance of fishes associated with submersed aquatic plants in the Potomac River. *North American Journal of Fisheries Management* 9: 101–111.

King, Ryan S., William V. DeLuca, Dennis F. Whigham, and Peter P. Marra. 2007. Threshold effects of coastal urbanization on *Phragmites australis* (common reed) abundance and foliar nitrogen in Chesapeake Bay. *Estuaries and Coasts* 30: 469–481.

Langeland, Kenneth A. 1996. *Hydrilla verticillata* (L. F.) Royle (Hydrocharitaceae), "The perfect aquatic weed". *Castanea* 61: 293–304.

Lissner, Jørgen, and Hans-Henrik Schierup. 1997. Effects of salinity on the growth of *Phragmites australis*. *Aquatic Botany* 55: 247–260.

Marks, M., B. Lapin, and J. Randall. 1994. Phragmites australis (P. communis): threats, management and monitoring. *Natural Areas Journal* 14: 285–294.

Martin, Laura J., and Bernd Blossey. 2013. The runaway weed: costs and failures of *Phragmites australis* management in the USA. *Estuaries and Coasts* 36: 626–632.

Martincic, J., V. Guberac, and S. Maric. 1997. Influence of winter rye (*Secale cereale* L.) seed size on germ and rootlet length, and grain yield. *Rost Vyroba* 43: 95–100.

Mateos-Naranjo, Enrique, and Susana Redondo-Gómez. 2015. Interpopulation differences in salinity tolerance of the invasive cordgrass *Spartina densiflora*: implications for invasion process. *Estuaries and Coasts* 39: 98–107.

Mauchamp, André, and François Mésleard. 2001. Salt tolerance in *Phragmites australis* populations from coastal Mediterranean marshes. *Aquatic Botany* 70: 39–52.

McCormick, Melissa K., Karin M. Kettenring, Heather M. Baron, and Dennis F. Whigham. 2010. Extent and mechanisms of *Phragmites australis* spread in brackish wetlands in a subestuary of the Chesapeake Bay, Maryland (USA). *Wetlands* 30: 67–74.

McNaughton, S.J. 1966. Ecotype function in *Typha* community-type. *Ecological Monographs* 36: 297–325.

McNaughton, S.J. 1975. R- and K-selection in *Typha*. *The American Naturalist* 109: 251–261.

Meyerson, Laura A., Kristin Saltonstall, Lisamarie Windham, Erik Kiviat, and Stuart Findlay. 2000. A comparison of *Phragmites australis* in freshwater and brackish marsh environments in North America. *Wetlands Ecology and Management* 8: 89–103.

Minchinton, Todd E., Juliet C. Simpson, and Mark D. Bertness. 2006. Mechanisms of exclusion of native coastal marsh plants by an invasive grass. *Journal of Ecology* 94: 342–354.

Mozder, Thomas J., and Patrick J. Megonigal. 2012. Jack-and-master trait responses to elevated CO_2 and N: a comparison of native and introduced *Phragmites australis*. *PloS One* 7: e42794.

Mozder, Thomas J., and Joseph C. Zieman. 2010. Ecophysiological differences between genetic lineages facilitate the invasion of non-native *Phragmites australis* in North American Atlantic coast wetlands. *Journal of Ecology* 98: 451–458.

Mozder, Thomas J., Joshua S. Caplan, Rachel N. Hager, C. Edward Proffitt, and Laura A. Meyerson. 2016. Contrasting trait responses to latitudinal climate variation in two lineages of an invasive grass. *Biological Invasions* 18: 2649–2660.

Neira, Carlos, Lisa A. Levin, and Edwin D. Grosholz. 2005. Benthic macrofaunal communities of three sites in San Francisco Bay invaded by hybrid *Spartina*, with comparison to uninvaded habitats. *Marine Ecology Progress Series* 292: 111–126.

Neira, Carlos, Edwin D. Grosholz, Lisa A. Levin, and Rachael Blake. 2006. Mechanisms generating modification of benthos following tidal flat invasion by a *Spartina* hybrid. *Ecological Applications* 16: 1391–1404.

Parker, I.M., D. Simberloff, W.M. Lonsdale, K. Goodell, M. Wonham, P.M. Karieva, M.H. Williamson, B. Von Holle, P.B. Moyle, J.E. Byers, and L. Goldwasser. 1999. Impact: toward a framework for

understanding the ecological effects of invaders. *Biological Invasions* 1: 3–19.

R Core Team. 2016. *R: A language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing http://www.R-project.org/.

R Studio Team. 2016. *RStudio: integrated development for R*. Boston: RStudio, Inc. http://www.rstudio.com/.

Ricciardi, Anthony, Martha F. Hoopes, Michael P. Marchetti, and Julie L. Lockwood. 2013. Progress toward understanding the ecological impacts of nonnative species. *Ecological Monographs* 83: 263–282.

Richards, Christina L., Oliver Bossdorf, Norris Z. Muth, Jessica Gurevitch, and Massimo Pigliucci. 2006. Jack of all trades, master of some? One the role of phenotypic plasticity in plant invasions. *Ecology Letters* 9: 981–993.

Ruiz, Gregory M., James T. Carlton, Edwin D. Grosholz, and Anson H. Hines. 1997. Global invasions of marine and estuarine habitats by non-indigenous species: mechanisms, extent, and consequences. *American Zoologist* 37: 621–632.

Ruiz, Gregory M., Paul W. Fofonoff, and Anson H. Hines. 1999. Non-indigenous species as stressors in estuarine and marine communities: assessing invasion impacts and interactions. *Limnology and Oceanography* 44: 950–972.

Ruiz, Gregory M., Paul W. Fofonoff, James T. Carlton, Marjorie J. Wonham, and Anson H. Hines. 2000. Invasion of coastal marine communities in North America: apparent patterns, processes, and biases. *Annual Review of Ecology and Systematics* 31: 481–531.

Saltonstall, Kirstin. 2003. A rapid method for identifying the origin of North American *Phragmites* populations using RFLP Analysis. *Wetlands* 23: 1043–1047.

Saltonstall, Kristin. 2002. Cryptic invasion by a non-native genotype of the common reed, *Phragmites australis*, into North America. *Proceedings of the National Academy of Sciences* 99: 2445–2449.

Saltonstall, Kristin, and J. Court Stevenson. 2007. The effect of nutrients on seedling growth of native and introduced *Phragmites australis*. *Aquatic Botany* 86: 331–336.

Sax, Dov F., John J. Stachowicz, James H. Brown, John F. Bruno, Michael N. Dawson, Steven D. Gaines, Richard K. Grosberg, Alan Hastings, Robert D. Holt, Margaret M. Mayfield, Mary I. O'Connor, and William R. Rice. 2007. Ecological and evolutionary insights from species invasions. *Trends in Ecology and Evolution* 22: 465–471.

Serafy, Joseph E., Riginal M. Harrell, and Linda M. Hurley. 1994. Mechanical removal of *Hydrilla* on the Potomac River, Maryland: local impacts on vegetation and associated fishes. *Journal of Freshwater Ecology* 2: 135–143.

Spencer, David F., and Gregory G. Ksander. 2006. Estimating *Arundo donax* ramet recruitment using degree-day based equations. *Aquatic Botany* 85: 282–288.

Stockwell, Craig A., Andrew P. Hendry, and Michael T. Kinnison. 2003. Contemporary evolution meets conservation biology. *Trends in Ecology and Evolution* 18: 94–101.

Strayer, David L., Valerie T. Eviner, Jonathan M. Jeschke, and Michael L. Pace. 2006. Understanding the long-term effects of species invasions. *Trends in Ecology and Evolution* 21: 645–651.

Talley, Theresa S., and Lisa A. Levin. 2001. Modification of sediments and macrofauna by an invasive marsh plant. *Biological Invasions* 3: 51–68.

Tepolt, C.K. 2015. Adaptation in marine invasions: a genetic perspective. *Biological Invasions* 17: 887–903.

Vasquez, Edward A., Edward P. Glenn, J. Jed Brown, Glenn R. Guntenspergen, and Stephen G. Nelson. 2005. Salt tolerance underlies the cryptic invasion of North American salt marshes by an introduced haplotype of the common reed *Phragmites australis* (Poaceae). *Marine Ecology Progress Series* 298: 1–8.

Weber, Ewald, and Bernhard Schmid. 1998. Latitudinal population differentiation in two species of *Solidago* (Asteraceae) introduced into Europe. *American Journal of Botany* 85: 1110–1121.

Whitham, Thomas G., William P. Young, Gregory D. Martinsen, Catherine A. Gehring, Jennifer A. Schweitzer, Stephen M. Shuster, Gina M. Wimp, Dylan G. Fischer, Joseph K. Bailey, Richard L. Lindroth, Scott Woolbright, and Cheryl R. Kuske. 2003. Community and ecosystem genetics: a consequence of the extended phenotype. *Ecology Ecological Society of America* 84: 559–573.

Williams, Susan L., and Edwin D. Grosholz. 2008. The invasive species challenge in estuarine and coastal environments: marrying management and science. *Estuaries and Coasts* 3: 3–20.

Zedler, Joy B., and Suzanne Kercher. 2004. Causes and consequences of invasive plants in wetlands: opportunities, opportunists, and outcomes. *Critical Review in Plant Science* 23: 431–452.