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Mechanisms of *Phragmites australis* invasion: feedbacks among genetic diversity, nutrients, and sexual reproduction

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Summary

1. A fundamental challenge to invasion ecology is to determine what factors cause an exotic species to spread rapidly long after the initial introduction. The increase of a resource (e.g. nitrogen) could trigger an expansion, but in other instances, species have overcome biological limitations (e.g. an Allee effect) like accumulating sufficient genetic diversity for successful reproduction. Understanding the ecological mechanisms governing plant invasions, such as nutrient enrichment or Allee effects, can be used to improve invasive plant management.

2. We used the invasive, introduced grass *Phragmites australis* as a model to evaluate the role of nutrient enrichment and Allee effects in invasion. Based on recent studies that demonstrated the importance of sexual reproduction in this plant's spread, we chose to focus our efforts on reproductive output. We examined the effects of patch-level genetic diversity on viable seed production across watersheds of the Chesapeake Bay, USA, with differing levels of anthropogenic development (a proxy for nutrient enrichment). In an outdoor mesocosm experiment, we treated *Phragmites* plants originating from forested and developed watersheds with elevated vs. ambient nutrients and cross vs. self-pollination and determined the effects on viable seed, floret and inflorescence production.

3. The proportion of viable seeds produced at field sites varied widely and was not directly related to watershed development. Instead, seed viability was positively related to patch-level genetic diversity, and patches in more developed watersheds had higher genetic diversity. Also, plants in larger patches produced a higher proportion of viable seeds. In the mesocosm experiment, seed viability was substantially higher for out-crossed plants. Elevated nutrients resulted in greater floret and inflorescence production, particularly for plants originating from developed vs. forested watersheds.

4. These findings have important management implications: small populations should be controlled before they accumulate sufficient genetic variation for prolific viable seed production, and landscape-scale nutrient management could further limit reproductive output.

5. *Synthesis and applications*. Our research shows how nutrient enrichment and a weak Allee effect can interact across multiple scales to impact invasion success and how understanding the ecological mechanisms governing plant invasions can be used to better inform invasive plant management.

Key-words: allee effect, anthropogenic development, common reed, cross-pollination, nitrogen, phosphorus, seed viability, superior genotypes

Introduction

A challenge to invasion ecology is to determine what factors cause an exotic species to become invasive, decades or centuries after introduction. A better mechanistic understanding of how species transition from a lag phase (*sensu* Crooks & Soulé 1999; Crooks 2005) to rapid population growth and spread is needed. Researchers have evaluated the role of human-caused global change (e.g. increased CO_2 levels, nutrient enrichment, altered hydrology) as the cause of this transition (Crooks

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2005). Others have focused on overcoming biological limitations that result in rapid spread (e.g. increased density that improves mate finding or a change in competitive ability that favours the invader; summarized in Crooks 2005). To develop a more sophisticated understanding of invasions, we need to know how multiple factors that interact across scales can govern the transition out of the lag phase.

Human impact on global nutrient cycles can benefit invasive species (Dukes & Mooney 1999; Galloway *et al*. 2008), and many native plant communities are susceptible to invasion by undesirable species under elevated nutrients (e.g. Huenneke *et al*. 1990; Ostertag & Verville 2002; Perry, Galatowitsch & Rosen 2004). Increased nutrient availability may change the outcome of competitive interactions to favour invaders, resulting in their rapid spread. Increased nutrients also increase reproductive output (e.g. Daehler 1998). The importance of nutrient-fuelled increases in reproductive output may have been historically underappreciated for largely clonal species, like grasses, because they enhance the invader's ability to establish new, genetically distinct populations in addition to enhancing their spatial dominance in already invaded habitats. In the light of increasing anthropogenic nutrient inputs, the links between nutrient enrichment and reproduction in invasive species require further investigation.

It is also possible that invasive species overcome a basic biological limitation. In recent years, the Allee effect, 'a positive relationship between any component of individual fitness and either numbers or density of conspecifics' (Allee 1931; Stephens, Sutherland & Freckleton 1999), has been examined as an element of invasive species. Historically, Allee effects have focused on rare and endangered species, but the implications for invaders cannot be ignored. Both 'weak' and 'strong' Allee effects can occur (Wang & Kot 2001; Taylor & Hastings 2005); both include a density-dependent component. Species that have a 'strong' Allee effect have negative population growth in small populations (e.g. rare or endangered species). Weak Allee effects are more likely to occur for invasive species where there is no risk of extinction at low densities, but where increased densities increase fitness and population growth rates (Taylor & Hastings 2005; Elam *et al*. 2007). Thus, Allee effects may explain why some invasive species exhibit a lag phase before rapid spread (Ackleh, Allen & Carter 2007). Mechanisms driving Allee effects in invasive plants include the potential for increased pollination success and seed set with increased density of conspecifics (Cappuccino 2004; Davis *et al*. 2004; Elam *et al*. 2007).

The introduced lineage of *Phragmites australis* (Cav.) Trin. *ex* Steud. (hereafter *Phragmites*) is invasive in wetlands across North America (Marks, Lapin & Randall 1994; Galatowitsch, Anderson & Ascher 1999; Meyerson *et al*. 2000; Saltonstall, Peterson & Soreng 2004). *Phragmites* was introduced from Europe more than a century ago, but has only recently become widespread and problematic (Saltonstall 2002). For instance, in a subestuary of Chesapeake Bay, USA, the number of patches and aerial extent of *Phragmites* increased 40× and 25×, respectively, since the early 1970s (McCormick *et al*. 2010a). Here, we use *Phragmites* as a model system to evaluate multiple interacting factors – nutrients and Allee effects – that may be responsible for the recent rapid spread of an invasive species.

Anthropogenic influences have been implicated in *Phragmites* spread, particularly through nutrient enrichment. In Chesapeake Bay, King *et al*. (2007) found that in estuarine wetlands in watersheds with more development (compared to forest-dominated watersheds), nitrogen levels were higher in the water and in *Phragmites* leaves, and *Phragmites* was more abundant. Links between eutrophication and *Phragmites* invasion also occur in New England, USA, salt marshes (Bertness, Ewanchuk & Silliman 2002; Silliman & Bertness 2004). Manipulative studies evaluating the mechanisms driving these relationships found that the invasive, introduced lineage of *Phragmites* performed better than native *Phragmites* and other native species under elevated nutrient conditions; invasive *Phragmites* had taller stems, and greater biomass and asexual reproduction (Minchinton & Bertness 2003; Rickey & Anderson 2004; Saltonstall, Peterson & Soreng 2004; Saltonstall & Stevenson 2007; Mozdzer & Zieman 2010). These studies suggest the importance of nutrient enrichment in *Phragmites* invasion. Recent research demonstrates the significance of *Phragmites* spread by seed (Belzile *et al*. 2010; Kettenring *et al*. 2010; McCormick *et al*. 2010a,b) and indicates that we need a better understanding of the interaction between nutrients and sexual reproduction.

Other factors, including overcoming biological limitations of small population sizes, are also likely to be important. McCormick *et al*. (2010b) suggested that *Phragmites*' recent success in Chesapeake Bay can be partially explained by an Allee effect owing to increased local levels of genetic diversity that promote viable seed production (i.e. increased fitness), although they did not measure the latter directly. Lambert & Casagrande (2007) indicated that self-compatibility is important in *Phragmites* invasion but Kettenring *et al*. (2010), in a small-scale field study, found that viable seed production was linked to local levels of genetic diversity, probably because increased patch-level genetic diversity favoured cross-pollination and viable seed production. Determining whether an Allee effect is occurring requires direct evaluation of the link between seed viability and genetic diversity on a larger scale, then confirming results with manipulative experiments.

We report results from a field study and an outdoor mesocosm experiment that evaluate the importance of interacting mechanisms on *Phragmites* sexual reproduction and invasion. We build on previous studies of *Phragmites* sexual reproduction and spread in one Chesapeake Bay subestuary and a large-scale analysis of the distribution of genetic variation within and among several subestuaries (Kettenring *et al*. 2010; McCormick *et al*. 2010a,b). We use findings from new research to demonstrate how *Phragmites* viable seed production is controlled by local levels of genetic diversity and the ability to cross-pollinate. We also show how nutrients affect reproductive output through floret and inflorescence production. Our findings complement the extensive literature on *Phragmites* rhizome spread (e.g. Hellings & Gallagher 1992; Amsberry *et al*. 2000; Bart & Hartman 2000, 2002, 2003; Minchinton &

Bertness 2003; Vasquez *et al.* 2005; League *et al.* 2006) and together provide a framework for understanding how *Phragmites* spreads locally and regionally.

Materials and methods

SEED VIABILITY FIELD STUDY

We evaluated *Phragmites* viable seed production and three potential factors that may affect reproductive output: anthropogenic development (a nutrient proxy), patch genetic diversity and patch size.

Study area

We studied brackish tidal wetlands in nine subestuaries of Chesapeake Bay with watersheds dominated by three land-use categories (King *et al.* 2005; Fig. 1). Forested watersheds had > 60% forest and < 15% commercial and residential development; mixed-developed watersheds had 15–50% development and developed watersheds had > 50% development. We chose subestuaries sampled by King *et al.* (2005, 2007) because of existing data on nutrient status. Parkers Creek was the only exception for which we did not have nutrient data. We assumed that *Phragmites* patches within each subestuary experienced comparable nutrient conditions because water in shallow Chesapeake Bay subestuaries is well mixed (Gallegos, Jordan & Correll 1992; Kettenring *et al.* 2010).

Defining and mapping patches

Each subestuary was divided into five segments of similar shoreline perimeter using aerial photographs. One *Phragmites* patch was selected per segment. We defined a *Phragmites* patch as a robust stand of plants isolated from others by ≥5 m or by ≥10 m if there were sparse *Phragmites* stems between robust stands. In each patch, we chose four sampling points ≥3 m into the stand where we collected leaves and seeds for viability and genetic analyses.

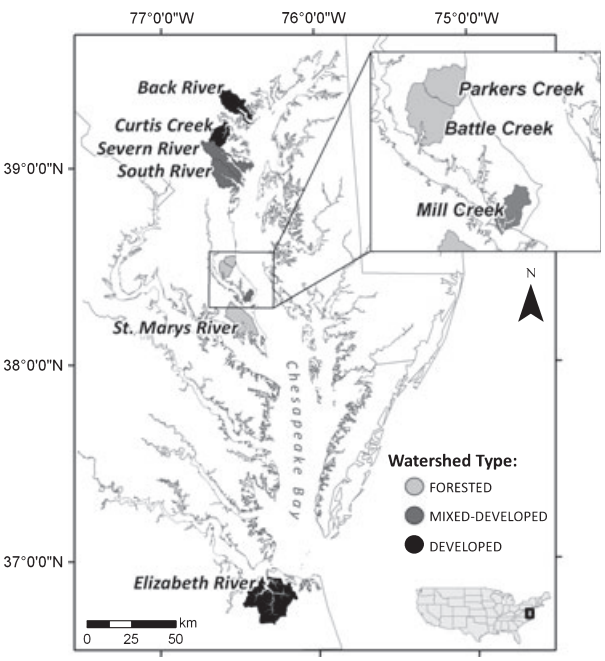

Fig. 1. The locations of the nine subestuaries in forested, mixed-developed and developed watersheds of Chesapeake Bay, USA.

We used a Global Positioning System (GPS) to determine the location and size of each patch by walking its perimeter, or if access was limited, we estimated size using GPS waypoints and delineating the stand on an aerial photograph. We digitized *Phragmites* patches in ARCGIS 9.2 (ESRI, Redlands, CA, USA) to determine patch size.

Seed collection and processing

We collected seeds from the five patches in each subestuary in fall, 2007. At each of the four sampling points per patch, we collected two inflorescences. In the laboratory, we manually stripped spikelets from each inflorescence. Seed viability was determined following the methods of Kettenring & Whigham (2009), which involved cold stratification at 4 °C, then seed incubation for 3 weeks at 19/7 °C (day/night temperature). We used our calculations of proportional germination as our measure of viable seeds because all ungerminated seeds were nonviable based on visual inspection.

Genetic variation assessment

To determine the number of genotypes within and among *Phragmites* patches in each subestuary, we collected leaf samples in the same locations as the seed collection (one leaf from each sampling point, total $n = 180$ leaves) and used microsatellite analysis following the methods of Saltonstall (2003). We stored the leaves at 4 °C until we extracted DNA from 20 mg of fresh tissue per leaf using a BioSprint 96 (QIAGEN, Inc., Valencia, CA, USA) following the supplied plant DNA extraction protocol.

We assessed multilocus phenotypes of individual *Phragmites* plants using microsatellite markers. We used eight primer pairs (developed by Saltonstall 2003) to target different DNA regions (Table 1 in McCormick *et al.* 2010a). These markers behave as unlinked within nonnative Chesapeake Bay *Phragmites* (unpublished data; McCormick *et al.* 2010b). We performed PCR amplification using a PTC-200 DNA Engine thermal cycler (MJ Research, Inc., Waltham, MA, USA), conditions and chemistry as per McCormick *et al.* (2010a).

We subjected amplicons to analysis on an ABI 3100 Automated Capillary DNA Sequencer (Applied Biosystems, Inc., Foster City, CA, USA) using a custom ROX (6-Carboxyl-×-Rhodamine) size standard to determine fragment sizes. After amplification, we combined PCR products with different fluorophores as per McCormick *et al.* (2010a). Fragment sizes were determined using GeneMapper v4.0 (Applied Biosystems, Inc.). Twenty samples were run in duplicate, generating an assignment error = 0·0025. We aligned fragments for all samples using a TRFLP peak sorting function for Excel (http://www.wsc.monash.edu.au/~cwalsh/treeflap.xls; Rees *et al.* 2004) and removed shadow peaks manually.

Analysis

To determine the effect of watershed type on viable seed production, we used a one-way ANOVA with watershed type as a fixed effect and

Table 1. Bruvo genetic diversity and number of genetic phenotypes (each presented as range; mean ± 1 SE) within patches in subestuaries with different amounts of watershed development

Watershed type	Diversity	Genetic phenotypes
Forested	0–0·114; 0·029 ± 0·009	1–4; 2·53 ± 0·31
Mixed-developed	0–0·149; 0·054 ± 0·012	1–4; 3·27 ± 0·27
Developed	0·007–0·099; 0·048 ± 0·007	2–4; 3·53 ± 0·19

subestuary number as a random effect nested within watershed type. We logit-transformed seed viability throughout the analyses to correct for the non-normal distribution of residuals. Genetic phenotypes were never repeated among patches, so we focused on genetic diversity within patches. Assuming out-crossing and given the allele frequencies we detected, the probability of the most common genotype arising by chance (the product of frequencies of all alleles in the genotype) was 0·00004; therefore, these loci should provide ample power to distinguish genotypes. Inbreeding could increase this probability but any of the less common alleles would reduce it. To determine the effect of within-patch genetic diversity on viable seed production, we conducted a regression of the proportion of viable seeds on patch-level Bruvo genetic diversity (Bruvo *et al.* 2004; natural log-transformed to correct for non-normality throughout the analyses) calculated between all pairs of sampled plants within each subestuary using GENODIVE (Meirmans & van Tienderen 2004). Bruvo genetic diversity is a metric that estimates the number of mutations separating two samples. It is designed for use with microsatellite markers and can handle mixed ploidy levels, as may be the case for *Phragmites*. We used a Pearson χ^2 test to determine whether the number of genotypes per patch differed in different watershed types. To determine the relationship between patch area and viable seed production, we used simple linear regression of proportion viable seeds on the natural log of patch area; proportional seed viability of all samples within a patch was averaged prior to inclusion in the analysis. To determine the effect of patch size on local levels of genetic diversity, we conducted a regression of patch-level Bruvo genetic diversity on patch area.

REPRODUCTIVE OUTPUT MESOCOSM EXPERIMENT

In an outdoor mesocosm experiment, we looked at the effects of cross- vs. self-pollination, ambient vs. elevated nutrients and plant source on *Phragmites* viable seed, floret and inflorescence production in a fully crossed factorial experiment. We included plant source as a factor in this experiment because we hypothesized that plants coming from forested watersheds might respond differently to nutrient enrichment than those coming from watersheds with more anthropogenic development.

Rhizome collection and preparation

We collected rhizomes from the field in March 2007, from one genetically distinct *Phragmites* patch (McCormick *et al.* 2010b) in each of four subestuaries of Chesapeake Bay: Battle Creek (BC), Saint Marys River (SMR), Severn River (SVR) and South River (SOR; Fig. 1). We chose two subestuaries in mixed-developed watersheds (SOR and SVR) and two in forested watersheds (BC and SMR) to allow comparisons between watershed types (see Analysis below). At each patch, we collected rhizomes from within a 1-m-diameter area to a depth of 30 cm. We trimmed the rhizomes to a standard length of two nodes and weighed them. Rhizomes were 5·5 g ± 1·0 (mean ± 1 SD). We stored the rhizomes for 2 months at 4 °C until the beginning of the experiment.

Experimental setup

We conducted the experiment 2007–2008 in an outdoor facility at the Smithsonian Environmental Research Center in Edgewater, MD (38°53·25′, −76°33·30′). In late May 2007, we planted nine randomly selected rhizomes from a single subestuary source into fibreglass bins (52 cm L × 43 cm W × 26 cm H), with 16 bins per subestuary for a total of 64 bins arranged in a completely randomized design. We filled the basins with a 60% sand, 40% soil mix by volume (Sunshine Basic Mix No. 2) and watered them regularly to maintain a moist substrate over the growing season. For each growing season, we added 60 mL of Instant Ocean® to the soil surface to supply micronutrients and achieve slightly brackish conditions in the tubs (≤5 ppt salinity). Approximately 2 months after planting the rhizomes, we thinned each bin, so that only one *Phragmites* plant remained for the rest of the experiment.

Fertilizer treatment

We chose two fertilizer treatments to approximate field conditions in forested vs. developed watersheds on Chesapeake Bay, based on published literature from the region (Jordan & Correll 1985; Jordan, Whigham & Correll 1989; King *et al.* 2007). We applied the ambient treatment (representing forested watersheds) of 4 g N m^{-2} $year^{-1}$ and 0·4 g P m^{-2} $year^{-1}$ as 1·41 g $(NH_4)_2SO_4$ and 0·06 g P_2O_5 bin^{-1} $month^{-1}$ to half of the bins during the growing season. To the other half of the bins, we applied the elevated treatment (representing developed watersheds) of 8 g N m^{-2} $year^{-1}$ and 0·8 g P m^{-2} $year^{-1}$ as 2·82 g $(NH_4)_2SO_4$ and 0·13 g P_2O_5 bin^{-1} $month^{-1}$. Nutrients were added in five monthly doses May–September 2007 and in five monthly doses March–July 2008 to coincide with plant emergence and end before anthesis. Each fertilizer treatment was spread over the soil surface and then watered.

Pollination treatment

In the second year of the experiment, nearly all experimental plants (62/64) produced inflorescences. We covered each inflorescence with a bag (REEMAY® Lawn and Garden Blanket Dupont) prior to anthesis and then subjected each plant to the pollination treatments: self-pollination or out-crossing in late August–early September 2008. We hand-pollinated one inflorescence per plant twice, 2 days apart, 1 day after pollen was first visible in the REEMAY bags and stigmas were receptive and then re-bagged them with REEMAY. Selfed inflorescences were hand-pollinated by shaking them in a clean pollinating bag (Lawson Showerproof'd Pollinating Bag, Northfield, IL, USA) to distribute pollen. Out-crossed inflorescences were hand-pollinated with a mix of pollen from plants originating from all four subestuaries. We obtained out-cross pollen by shaking one nontarget inflorescence from each subestuary into a pollinating bag and then placing it over the target inflorescence and shaking it. All inflorescences that were crossed on a given day received pollen from the same collection bag. We waited for 2 weeks to ensure no stigmas remained receptive and then removed the REEMAY bags to allow seed maturation. We counted the number of inflorescences produced in each bin to determine reproductive effort on a per-plant basis. We harvested all target inflorescences 6 weeks after pollination when seeds appeared mature.

Seed viability analysis

We manually removed the florets from each of the hand-pollinated inflorescences to evaluate seed viability. We dissected three subsamples of 50 florets per inflorescence for each of the 62 plants and categorized them as viable (robust caryopsis with endosperm) or nonviable (shrivelled or lacking caryopsis). This direct assessment of seed viability, compared with the field study, was chosen for efficiency given the smaller number of seeds being processed; a pilot study indicated that these methods were comparable (Kettenring, unpublished data).

To estimate the number of florets per inflorescence, we first determined the weight of all 62 inflorescences. For each population ×

nutrient × pollination treatment combination, we randomly chose one inflorescence, took five samples of florets, weighed them and counted the number of florets in the subsample. This information was used for our simple linear regression analysis of inflorescence weight vs. the number of florets in the subsample. These equations accurately predicted the number of florets in a subsample (R^2 = 0·82–0·99) and were used to estimate how many florets were produced by each of the 62 inflorescences.

Analysis

To evaluate the effects of nutrients, pollination type and watershed type on viable seed production, we analysed our data as a three-way factorial experiment in a split plot design. The whole plot factor was watershed type (forested or mixed-developed), and the whole plot unit was subestuary (a random effect). The subplot factors were nutrients and pollination type, and the subplot unit was seeds from the group of plants subjected to a unique treatment combination. We made multiple comparisons of means using Tukey's HSD for these and subsequent ANOVA analyses, and we assessed them at α = 0·10 to increase power (Day & Quinn 1989).

To evaluate the effects of watershed type and nutrients on the number of inflorescences produced per plant, we conducted a two-way factorial experiment in a split plot design. The whole plot factor and unit were identical to above. The subplot factor was nutrient level, and the subplot unit was inflorescences from the group of plants subjected to a unique treatment combination. Similarly, we used a nearly identical ANOVA structure to test the effects of subestuary type and nutrients on the number of florets per inflorescence but the number of florets produced per plant was natural log-transformed.

Results

SEED VIABILITY FIELD STUDY

Watershed type did not have a significant effect on viable seed production (Fig. 2; F = 1·4; d.f. = 2, 6; P = 0·32). However, patches with greater genetic diversity produced more viable seeds (Fig. 3). The number of genotypes per patch differed significantly among the three watershed development classes; patches in mixed-developed and developed watersheds contained more genotypes per patch and had higher Bruvo genetic diversity (Pearson χ^2 = 17·8, P = 0·007; Table 1).

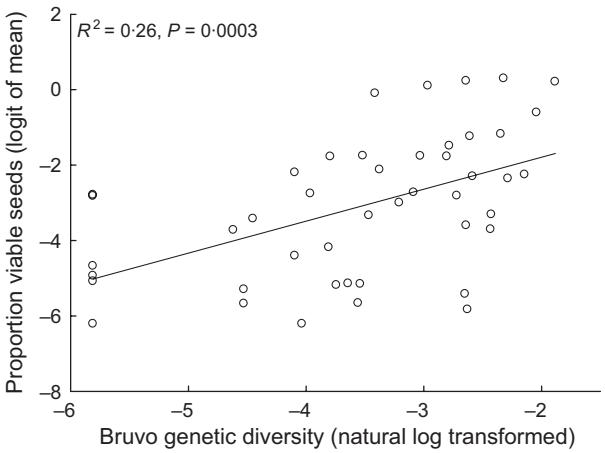

Fig. 3. The relationship between patch-level *Phragmites* viable seed production and Bruvo genetic diversity.

We found a weak positive relationship between patch area and viable seed production (Fig. 4); more viable seeds per plant were produced in larger patches. There was no significant relationship between the size of the patch and the amount of genetic diversity within a patch (R^2 = 0·03, P = 0·30).

REPRODUCTIVE OUTPUT MESOCOSM EXPERIMENT

Pollination treatment drove viable seed production in *Phragmites*. Cross-pollinated plants produced significantly more (almost 10×) viable seeds than did self-pollinated plants (Fig. 5; F = 80·0; d.f. = 1,6; P = 0·0001). Subestuary type (F = 1·2; d.f. = 1,2; P = 0·39) and nutrients (F = 1·1; d.f. = 1,6; P = 0·33) did not significantly affect viable seed production. However, *Phragmites* produced significantly more inflorescences per plant when subjected to elevated vs. ambient nutrients (1·7× as many; Fig. 6a; F = 20·9; d.f. = 1,10; P = 0·001) and when the rhizomes originated from a

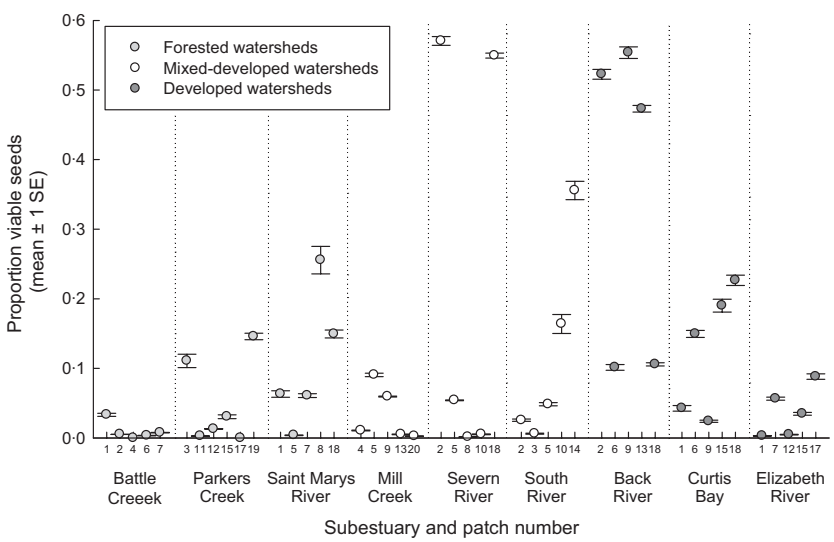

Fig. 2. *Phragmites* viable seed production of five patches in each of three subestuaries in each of three watershed types in Chesapeake Bay.

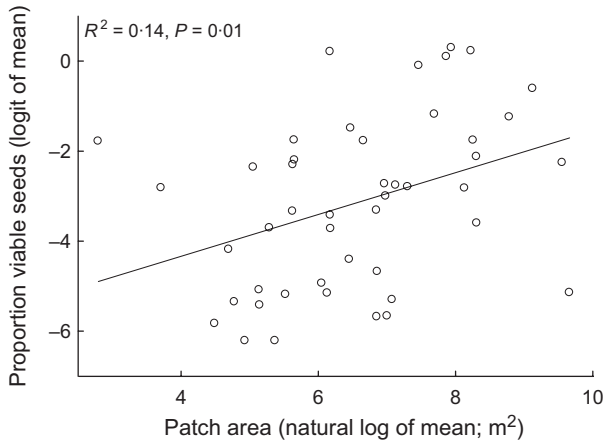

Fig. 4. The relationship between the size of *Phragmites* patches and the proportion of viable seeds that *Phragmites* produces.

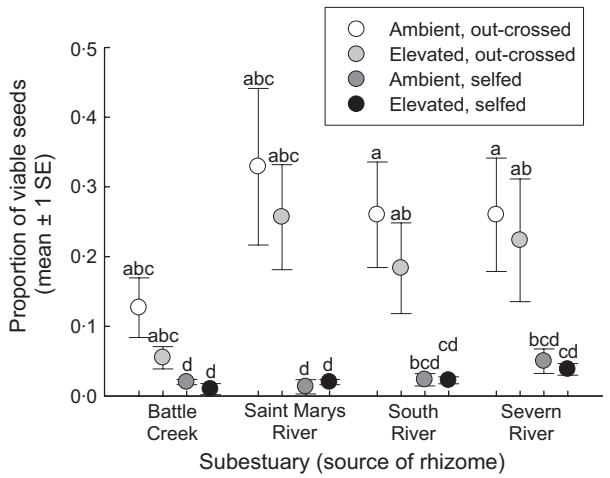

Fig. 5. The effects of pollination type, nutrients and watershed development surrounding subestuaries (Battle Creek and Saint Marys River have forested watersheds; South and Severn Rivers have mixed-developed watersheds) on viable seed production.

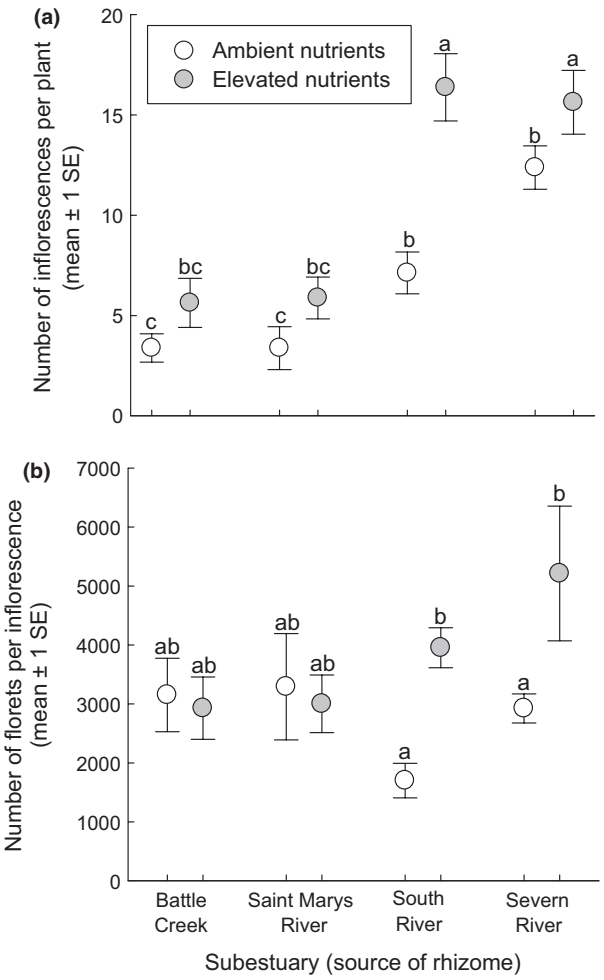

Fig. 6. The effects of nutrients and watershed development surrounding subestuaries (Battle Creek and Saint Marys River have forested watersheds; South and Severn Rivers have mixed-developed watersheds) on (a) inflorescence and (b) floret production.

subestuary with mixed-development (2·4× as many; F = 54·4; d.f. = 1,2; P = 0·02). Nutrients had a marginally significant effect on the number of florets produced per inflorescence (1·4× as many; Fig. 6b; F = 4·0; d.f. = 1,10; P = 0·07). There was also a significant interaction between nutrients and watershed type (F = 5·6; d.f. = 1,10; P = 0·04), indicating that plants originating from subestuaries in mixed-developed but not forested watersheds were affected more by increased nutrients (1·6× as many florets produced). Overall, cross-pollination and elevated nutrients on plants originating from developed watersheds resulted in 35× as many viable seeds being produced per plant, compared with those that were self-pollinated, subject to ambient nutrients and originated from forested watersheds.

Discussion

We describe two interacting mechanisms that are likely to be important in the recent rapid invasion of *Phragmites* in Chesapeake Bay: (i) *Phragmites* has accumulated sufficient genetic diversity in many subestuaries for abundant viable seed production (i.e. a weak Allee effect); (ii) increased nutrients benefit *Phragmites* floret and inflorescence production, especially for plants originating from more developed watersheds. These combined factors can contribute to greater reproductive output of *Phragmites*, which can result in rapid spread and a positive feedback. In this feedback, as more viable seeds are produced, *Phragmites* can increase local levels of genetic diversity and thus viable seed production. In wetlands with higher nutrients, *Phragmites* seedlings and adult plants grow larger and produce more stems, florets, inflorescences and thus seeds, further benefiting the invasion (this study; Minchinton & Bertness 2003; Rickey & Anderson 2004; King *et al.* 2007). These findings, together with the abundant literature on *Phragmites* reproduction via rhizomes and our previous work on *Phragmites* in Chesapeake Bay, suggest that reproduction and spread by seed is important both locally and regionally, while clonal integration allows *Phragmites* to expand locally once established (Amsberry *et al.* 2000; Bart & Hartman 2000,

2002, 2003; Minchinton & Bertness 2003; McCormick *et al.* 2010a,b).

Phragmites can serve as a model system to study weak Allee effects related to self-incompatibility in invasive plants. Given the substantial sexual reproduction occurring in Chesapeake Bay *Phragmites* and the resulting continuum of relatedness (McCormick *et al.* 2010a,b), a logical next step is to determine whether genotypes differ in the relatedness required for out-crossing, as was found for *Raphanus sativus* (wild radish; Elam *et al.* 2007). Further, additional research should investigate whether the requirement for out-crossing and variation in the availability of out-crossed pollen affects where and when out-crossing occurs (Levin, Kelley & Sarkar 2009). If so, there may be different Allee phenomena that drive the spread of *Phragmites* over space and time, as has been shown for *Lymantria dispar* (gypsy moths; Tobin *et al.* 2006). *Phragmites* seeds probably disperse widely, but newly emerging plants may not be able to reproduce sexually because of a lack of out-crossed pollen (Levin, Kelley & Sarkar 2009). In fact, *Phragmites* may be seed limited during its initial invasion into an area, but this hypothesis has not been tested. The accumulation of genetic diversity may result from the same alleles being rearranged into new multilocus genotypes through sex and recombination or from new genotypes or alleles arriving in invaded areas. Regardless of the source of genetic variation, its consequences depend, in part, on the requirements for out-crossing.

Previous work in Chesapeake Bay found that *Phragmites* was more abundant in subestuaries, where the watershed had greater anthropogenic development (King *et al.* 2007). One potential explanation for this phenomenon is that higher nutrients favour *Phragmites* sexual reproduction and hence expansion. Here, we show that *Phragmites* produces more florets and inflorescences under higher nutrients, particularly for plants originating from developed watersheds. These findings are similar to those of Daehler (1998) for *Spartina alterniflora* in San Francisco Bay, CA, USA, where nutrient-enriched plants produced larger and more numerous inflorescences but nutrients did not affect seed set. Subsequent *Phragmites* seedling emergence and establishment probably also benefit from increased nutrients (Saltonstall & Stevenson 2007) and more disturbances associated with anthropogenic development that create open space for plants to thrive. These factors have not been evaluated in the field to determine what conditions are required for *Phragmites*' early life stages. Few studies have observed *Phragmites* seedlings in the field (but see Alvarez, Tron & Mauchamp 2005; Brisson, Paradis & Bellavance 2008), and factors controlling seedlings remain an important area of research.

The positive effects of nutrients on reproductive output of *Phragmites* are not too surprising given ecological theory suggesting that resource availability is a critical factor controlling invasions (Davis, Grime & Thompson 2000) and that in restoration applications, many invasives can be controlled by nutrient impoverishment (e.g. Averett *et al.* 2004; Perry, Galatowitsch & Rosen 2004). However, the most interesting finding from this study is that there are *multiple* interacting factors affecting *Phragmites* invasion, not simply links to nutrient enrichment, suggesting new theoretical questions and management challenges. Our findings indicate that, much as in the invasion of *S. alterniflora* (Daehler & Strong 1994), some *Phragmites* patches are likely contributing significantly more seeds than other patches because of differences in genetic and nutrient effects. Future studies should address how variation in genetic diversity among patches and variation in nutrients among subestuaries interact across different scales to drive variable reproductive output of *Phragmites*. Taking these findings one step further, we suggest that elevated nutrients could actually alter the weak Allee effect for *Phragmites*; the quantity of seed production would be higher with elevated nutrients even if seed viability would be limited by a lack of pollen from different genotypes.

It will be important to understand how variable reproductive output influences the spatial pattern of *Phragmites* invasion and drives changes in *Phragmites* gene frequencies over time (Daehler & Strong 1994; Daehler 1998). To date, no research has assessed whether there is any selective colonization or postcolonization selection of *Phragmites* genotypes particularly suited to local environmental conditions. Interestingly, we found that genotypes of *Phragmites* derived from mixed-developed watersheds in our mesocosm experiment responded more strongly to nutrient enrichment than those from forested watersheds and so would be expected to contribute more to invasion. The potential genetic basis of this response requires further investigation.

Understanding the mechanisms favouring the spread of invasives is essential because it can inform under what conditions introduced populations may become problematic (Crooks 2005) and can aid management. The findings presented here and from our other research can be used to further management discussions:

1. We suggest that it is important to control and eradicate small, satellite patches (Moody & Mack 1988; Cappuccino 2004) of *Phragmites* before they accumulate sufficient genetic variation to produce viable seeds, in addition to controlling larger, more productive patches (larger *Phragmites* patches produce more viable seeds; this study; Kettenring *et al.* 2010). Although some patches may produce few seeds, the lag phase of an invasion can give managers a 'false sense of security'; the patch may not have yet acquired the diversity required to produce abundant viable seeds (Davis *et al.* 2004; Taylor & Hastings 2005). Complete local eradication (within the distance travelled by dispersing seeds or pollen) may be the only successful approach to long-term management in nutrient-rich estuarine wetlands.

2. Removal and low-cost long-term management of *Phragmites* in targeted forested watersheds should become another management approach. While the invasion of *Phragmites* is widespread throughout Chesapeake Bay (King *et al.* 2007), some predominantly forested watersheds have few patches and eradication is still possible. Management at the subestuary scale is important because most genetic exchange occurs within subestuaries (McCormick *et al.* 2010b). Long-term control requires the removal of pollen and seed sources within individual subestuaries.

3. The importance of nutrients for sexual reproduction in *Phragmites* suggests that there are landscape factors controlling its invasion, factors that are often beyond managers' control and require larger-scale coordination. Nutrient management will be essential for *Phragmites* control (Minchinton & Bertness 2003; Rickey & Anderson 2004; Packett & Chambers 2006).

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