Restoration Potential of Phragmites-Dominated Wetlands in Chesapeake Bay: Interactions between Disturbance, Nutrients, and Genetic Diversity

Introduction

Invasive species are currently one of the greatest threats to native ecosystems worldwide (1). Invasive plants infest over one hundred million acres of land in the U.S. alone (2) and wetlands are more prone to invasion than other ecosystems (3). Many plant invaders can alter an ecosystem by creating an alternative stable state that prevents original plant communities from reestablishing once the invader is removed (4). I am investigating the impacts of removing a keystone wetland invader (*Phragmites australis)* on plant communities and the environment, and how herbivores impact recruitment and clonal diversity in an invasive grass to increase knowledge on the science of invasion and improve restoration outcomes.

An invasive European lineage of *Phragmites australis* (*Phragmites*) has rapidly expanded its range into North American wetlands over the last five decades (5). *Phragmites* is an ecological engineer; it alters the hydrology and nutrient availability of wetlands (7,8,9,10) resulting in up to a five-fold decrease in species diversity (6). The major component of my doctoral research is a large-scale manipulation that examines the interplay of nutrients, disturbance, and sexual reproduction on the management of *Phragmites* across the Chesapeake Bay in collaboration with colleagues at the Smithsonian Environmental Research Center (SERC), Utah State University (USU), and several other institutions. I am applying for the Garden Club of America Coastal Wetland Scholarship to help complete the fourth year of this study.

The recent expansion of *Phragmites* has been connected to human land-use practices that disturb wetlands and increase nutrient availability (11,12,13). However, the impact of land-use on restoration outcomes is a major knowledge gap, and the environmental conditions that promote *Phragmites*' success are still unclear. Studies reporting the composition of plant communities that return when *Phragmites* is removed are rare (14) and studies on invasive plants seldom report more than two years of post-removal data (15).

Efforts to control *Phragmites* may have unintended consequences. *Phragmites* removal is likely to create the disturbed conditions thought to promote seedling establishment by the reed as its seeds can recolonize areas denuded by *Phragmites* removal. Historically, *Phragmites* was thought to spread primarily by clonal growth (16). Recently, SERC scientists found that sexual reproduction is more prevalent in its spread than previously thought and new patches are likely colonized by seed (17,18). Human disturbances, as one would find in more developed watersheds, allow for germination, thereby increasing genetic diversity in *Phragmites* populations (17,18), and nutrient runoff increases seed production (19). As genetic diversity increases, *Phragmites* stands produce more viable seeds, which in turn increases local levels of *Phragmites* genetic diversity in a positive feedback loop (19).

The system-wide impacts of control methods such as herbicide use are unclear (14), as is the efficacy of these methods in returning vegetation to a pre-invaded state. By identifying the specific physical conditions associated with disturbance (nutrients, salinity, hydrology, *etc.*) that promote, or result from, *Phragmites* invasion, we can minimize unintended consequences of control, restore habitat, and work toward preventing future invasions. My doctoral research addresses this need by tracking plant communities and environmental changes in marshes that have *Phragmites* removed in watersheds with differing levels of human development.

Research Questions

1) Does *Phragmites* removal by herbicide allow native plant communities to recolonize areas where they were displaced by *Phragmites*?
2) Does *Phragmites* removal allow the marshes to return to the physical conditions found in the reference marshes?
3) Does the seedbank differ between native marsh and the *Phragmites* removal and control sites and how does it change with time since herbicide treatments?
4) Does *Phragmites* removal act as a disturbance, fostering increased genetic diversity and sexual reproduction in recolonizing *Phragmites* populations?
5) Is there a connection between watershed-scale land use and the likelihood that a marsh will recover from *Phragmites* invasion (physical and biotic environments) after herbicide treatments?

Methods

I am the lead researcher for the described work, but all methods are in collaboration with the Whigham Lab at SERC and the Kettenring Lab at USU. We survey vegetation and wetland recovery after *Phragmites* removal in 8 Chesapeake Bay subestuaries from 3 different land-use categories (n=3: urban, forested; n=2 agricultural; per 11) annually from 2011-2015. In each marsh, we record changes in vegetation (visual estimates percent cover) and nutrients (mixed bed ion exchange resins) between sections where *Phragmites* is removed, left intact, and a reference marsh of native vegetation at 5 quadrats, along each of 3 permanent transects in each marsh section. In the *Phragmites* removal sections, the reeds were treated with glyphosate by aerial spraying in October 2011 and completed follow up treatments in 2013 (3 treatments total). Stem density, basal diameter of a subset of stems, flowering and herbivory rates, as well as seed germination rates, determine *Phragmites* vigor. I am in the process of analyzing the first 3 years of plant community and nutrient data and anticipate results by February 2014.

We sample the soil seed bank prior to each growing season in order to determine if there is a sufficient native seed bank to promote reestablishment of the native vegetation and to determine if there is a decrease in *Phragmites* seeds after herbicide treatments. We harvest seed bank cores (n=15 per treatment within each marsh, associated with permanent quadrats) each March and cold stratify the seed bank samples for 6 months at SERC. I germinate the cores in a greenhouse at USU using the seedling emergence method (20). Preliminary results indicate that there are differences between subestuaries, but not treatments, implying that the seedbank is transported by tides and is not dependent on the vegetation canopy.

We are tracking the spread of individual *Phragmites* clones and establishment of new seedlings from 2011 through 2015 (2 seasons post spraying). By taking tissue samples along each transect, we can map the location of each clone in a stand and determine the number and relative size of the clones. Individual clones are identified by microsatellite analysis (per 17) and changes in clonal richness will be tracked between treatments for the duration of the study.

Budget and Justification

I am seeking partial support for travel from Utah State University to SERC by personal vehicle. I need to drive each field season in order to transport equipment and samples between USU and my field sites. I am also requesting a budget for materials to conduct the microsatellite analysis for the fourth consecutive year. See **Table #1** for a detailed materials budget.

Timeline and Progress (based upon dissertation defense in May of 2016):

This study began in 2010, and has been progressing according to schedule since. My detailed timeline and progress on all research questions can be found in **Table #2**. I have a minimum of 3 years data for all of the research questions detailed above, and am preparing the pretreatment data for publication in Spring of 2014. I am currently analyzing the 2011-13 data for presentation at the Joint Aquatic Science Meeting this coming May. The studies described here will result in a planned minimum of 3 peer reviewed manuscripts, in addition to at least 1 conference talk per year (Society of Wetland Scientists, or Coastal and Estuarine Research Federation, depending on year).

Conclusions

The research proposed here will further the science of wetland management and improve our knowledge of the ecology of biological invasions. *Phragmites* is an elegant model species for studying the ecology of invasive species, especially keystone invaders that form stable states. Human alteration of wetlands is known to facilitate invasion by Phragmites and the proposed study will help guide future land-use, management, and restoration decisions. Large-scale manipulations, such as our *Phragmites* removal project in the Chesapeake Bay, provide excellent opportunities to determine the biological and environmental factors that promote invasion across multiple watersheds. Current control practices are necessarily aggressive and may have unintended consequences on wetland environments, possibly even promoting further invasion and maintaining *Phragmites'* genetic diversity and its ability to produce abundant viable seed. My results will assist managers in identifying wetlands that are most likely to benefit from restoration by determining the physical conditions and land-use patterns that are associated with invasion and recovery. This will allow restoration efforts to be streamlined in areas that are likely to return to their pre-invasion state, ensuring that restoration dollars directly increase habitat quality and recovery of biodiversity.

Works Cited

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Table 1. Requested Support	2014
Travel	
UT to MD by car (partial support for round trip)	***$2,000***
Expendable Materials	
Taq Polymerase	$600
DNA Extraction Kits	$1,600
PCR Plates	$800
Subtotal	***$3,000***
Total Support Requested from Garden Club of America	**$5,000**

Table 2. Timeline and Progress					
Task	**2011**	**2012**	**2013**	**2014**	**2015**
Herbicide treatment	**√**	**√**	**√**	**---**	**---**
Collect seed bank samples	**√**	**√**	**√**	**---**	**---**
Install/harvest nutrient resins	**√**	**√**	**√**	**June/ August**	**June/ August**
Collect vegetation and salinity data	**√**	**√**	**√**	**July-August**	**July-August**
Collect molecular samples	**√**	**√**	**√**	**July-August**	**July-August**
Germinate seed banks	**√**	**√**	**√**	**September**	**---**
Analyze molecular samples	**√**	**√**	**Winter**	**Winter**	**Winter**
Analyze nutrient resins	**√**	**√**	**√**	**Winter**	**Winter**
Collect flowering and herbivory data	**√**	**√**	**√**	**October**	**---**
Collect inflorescences and seed for germination	**√**	*****	**√**	**November**	**---**
Germination tests	**√**	*****	**May**	**May**	**---**
Talks, workshops, symposia coauthored	**8**	**3**	**7**	**1 (accepted) 2 (anticipated)**	**TBD**
Manuscripts submitted			**3**	**2 (in prep) 2 (anticipated)**	**TBD**
Manuscripts accepted			**3**	**TBD**	**TBD**
*Cancelled due to Hurricane Sandy					