APPENDIX 8

Experimental Application of Invasive Plant Removal Techniques for the Control of Non-Native *Phragmites australis* in the Anacostia River in Washington D.C.

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COVER PAGE:
Two photos taken by Anacostia Watershed Society interns depicting before (left, 2013) and after (right, 2014) control on Heritage Island Marsh in Anacostia Park, Washington D.C.

Experimental Application of Invasive Plant Removal Techniques for the Control of Non-Native *Phragmites australis* in the Anacostia River in Washington D.C.

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The Anacostia Watershed Society (AWS) has produced this study with the goal of conveying vital wetland restoration information to the scientific community, land managers, invasive species removal specialists, and the interested public. This article has been peer-reviewed for the sake of scientific accuracy, and professional purpose.

The Anacostia River Watershed encompasses 176 square miles including the eastern portion of Washington D.C. as well as large portions of Prince Georges and Montgomery County. Through the processes of agriculture and urbanization the Anacostia River has been intensely degraded. In the 1980's the Anacostia River was determined hopelessly polluted. AWS has been working to change this opinion since 1989.

AWS is committed to the restoration and reclamation of the once thriving Anacostia River. For the past twenty five years the Anacostia Watershed Society has been a leading force in shifting not only the health, but also the negative mentality associated with the River. The Anacostia Watershed Society's mission, in keeping with the clean water act issued by the Environmental Protection Agency, is to produce a fishable and swimmable river by 2025; however, this daunting task requires scientific, business, educational, and community efforts. AWS attempts to reach these avenues of change through our four different departments: Public policy and Advocacy, Stewardship, Education, and Recreation.

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Abstract

Non-native invasive *Phragmites australis*, when present in a monotypic stand, reduces the functionality and biodiversity of marshes in the Anacostia River and the Atlantic region. Due to *Phragmites australis'* ability to spread vigorously through rhizomes and diminished presence of biological controls, *Phragmites* outcompetes existing native vegetation, forming monocultures, which are not conducive to the naturally evolved wildlife of the region (Cronin). *Phragmites* populations were identified in the tidal emergent regions of the Anacostia Wetlands, and three transects with four randomly assigned plots per transect were established per treatment, twelve treatments total, including controls. In the summer of 2013 through the fall of 2014, an ocular estimation of percent *Phragmites* cover before and after the application of various management techniques took place within the Anacostia River, along the Fringe wetlands and Heritage/Kingman Marsh, to document the efficiency of various control methods. Plots were assessed by site (Fringe or Kingman), mowing treatment (mowed or 'unmowed'), and herbicide treatments (no herbicide, Habitat, Rodeo, and Rodeo/Habitat mix). Percent change in non-native *Phragmites* coverage was determined from the pre (2013) and post (2014) control *Phragmites* surveys, and a logistic regression was used to determine the probability of a successful removal, defined as a ≥75% reduction. A logistic regression was used due to a lack of normality within the data. Non-*Phragmites* coverage, which consists of any plant species other than *Phragmites,* was also monitored in order to determine the resurgence of wetland plants following a *Phragmites* control method. Non-*Phragmites* coverage was assessed using a repeated measure analysis of variance (ANOVA) because no statistical assumptions were violated with the non-*Phragmites* data. The synthesized data specify that site variations, between a low marsh and high marsh, have statistically different outcomes when preforming a removal of ≥75% ($p = 0.0076$), where removal was more effective in a low marsh. Likewise, mowing prior to an application of herbicide significantly decreases the success of removal, ($p = 0.0000$). Lastly, the data from different herbicide treatments statistically infer that Habitat has no notable influence upon the removal of *Phragmites* when compared to the plots that received no herbicide in the Anacostia wetlands ($p = 0.2313$). Conversely, Rodeo generated a significantly higher probability of success (a ≥75% removal), with a p-value of 0.0003, signifying Rodeo's increased efficiency over Habitat. A Rodeo and Habitat mix was also applied, producing a p-value of 0.0000 when compared to no herbicide, dictating that the mix caused a significant reduction in *Phragmites* post treatment. However, upon a secondary logistic regression comparing Rodeo and Rodeo-Habitat mix, it was concluded that the difference cannot be statistically supported, suggesting that the marked decrease from the mix may have been a result of the Rodeo interaction. Non-*Phragmites* data, highlighting the resurgence of wetlands plants, were measured, but showed no significance between site or mow treatment. Herbicide did exhibit significance, but only from the Rodeo-Habitat mix when compared to the remaining herbicide treatments, potentially indicating the increase efficiency of the Rodeo-Habitat mix as a control mechanism.

Introduction

Phragmites australis has been a native to the mid-Atlantic region for a considerable period of time, archeological evidence has suggested over 40,000 years (Swearingen 2010). However, European genotypes, which are substantially more competitive than the native counter-part, have implanted across the United States (Swearingen 2010). Non-native *Phragmites australis* is a monoculture producing invasive that exploits and excludes the native flora within a given area (Capotosto and Wolfe 2007, Kaufman and Kaufman 2007). "To generate a monoculture, the invader [*Phragmites*] must garner more resources than resident natives and, once established, persist despite high densities of conspecific neighbors," establishing a non-native ecosystem that is challenging to eradicate (Bertness 2011). The non-native *Phragmites* species, spreading through seed and underground rhizomes, can produce dense and tall colonies (up to 15 feet), which strangle the native vegetation and alter the existing ecosystem structure and species composition (Swearingen 2010). Once a monoculture is established, non-native *Phragmites* drastically affects the area's hydrology through the build-up of sediments and rhizome interactions, reducing food sources and ecosystem function (Swearingen 2010).

The Anacostia River contained roughly 1,000 hectares (~2,500 acres) of natural non-tidal emergent wetlands between the 1600's to the 1880's. Through land use changes this number has dwindled to only four percent of the original, less than 40 hectares remain (Baldwin 2004). Yet, restoration efforts have increased these degraded wetlands to nearly 180 acres, ~72 hectares (Anacostia). In congruence, the wildlife that relies upon these crucial habitats has declined as a result of reduced habitat and food sources. Thus, it is imperative that the wetlands the Anacostia River currently possesses must be as fully functioning and diverse as possible to recover the native biota. *Phragmites australis,* through its voracious, monoculture forming habit, directly limits the species richness and practicality of the wetlands in the Anacostia; in result, the control of *Phragmites* to more reasonable levels is critical to the restoration process of the Anacostia as well as other degraded wetlands (Cross 1989).

Due to the competitive nature of the introduced *Phragmites* genotypes on the east coast, there has been an increasing need to understand the efficiency of different removal techniques. Of the typical methods used within the region, mowing and herbiciding, this study attempts to account for the effectiveness of the various treatments, and determine the most successful method of control. With the intention of having this knowledge spread, allowing for *Phragmites* Australis removal operations to be accomplished in the most efficient manner.

Kingman Marsh was once an extensive tidal marsh dominated by Wild Rice (*Zizania aquatica*). At the beginning of the 19th century, the area was a famous destination for Sora rail hunters. In the 1920's and 1940's, the U.S. Army Corps of Engineers (USACE) dredged and filled wetlands in an effort to control malaria. The straightening of the river and construction of a sea wall by the Civilian Conservation Corps negatively affected the river's tidal emergent wetlands. Since then, sedimentation and the Resident Canada Geese grazing impacts have been two large stressors for these tidal wetlands. For the last 20 years, efforts have been made to convert these tidal mudflats into marsh. Revegetation and the installation of goose exclosures to prevent goose grazing have been carried out in this area by AWS, NPS, USGS and USACE. The 6-hectare (ha) River Fringe Wetlands were reconstructed along the main stem of the Anacostia River in Washington, DC during the summer of 2003 (Krafft *et al*., 2009). The Fringe Wetlands consist of two separate planting cells. Fringe A, located adjacent to Lower Kingman Island (west bank of the Anacostia), has an area of 1.6 ha; Fringe B, located on the east bank of the Anacostia River, occupies 4.4 ha (Krafft *et al*., 2009). For the creation of the River Fringe wetlands, dredged sediment materials were placed to increase elevations to support emergent vegetation. Fencing, stringing and flagging was installed to prevent goose grazing and native herbaceous emergent plant species were planted.

The project site is located in Kingman marsh and the River Fringe wetlands in NE Washington, DC, west and East of Kingman Island. Administratively, the site belongs to Anacostia Park, a park owned by the National Park Service (NPS)/ National Capital Parks-East (NACE). The area in focus for the purposes of this study is the area between Benning Rd NE and East Capitol Street NE.

Phragmites australis removal from these selected regions took place under six different treatments, with various trials, and the presence of two controls. Two herbicides were selected that are typically used to combat European *Phragmites*: Rodeo (R), Habitat (H) and a combination of the two Rodeo-Habitat (R-H) were selected. Of these herbicide treatments, a physical removal, mowing, took place prior to the spraying in a portion of the plots in order to determine if mowing or not mowing (unmowed) had a significant effect on the management of the invasive.

Results indicate that the plots left 'unmowed' were more severely affected by the herbicide, and the Rodeo-Habitat mix was the most effective, followed by Rodeo, which is contradictory to most of the conventional practices, suggesting that Habitat is more effective. The significance of these results will be discussed further in the discussion.

Location

The control effort occurred along the tidal Anacostia River Fringe and adjacent Lower Kingman Marsh, specifically in Kingman Marsh Area 2 and Heritage Island Wetland as well as Anacostia River Fringe B adjacent to River Terrace (Figure 1). A small patch of *Phragmites* will be targeted below East Capital Street on the Anacostia Fringe A adjacent to Lower Kingman Island as well (Figure 1). For treatment naming purposes, all plots located on Kingman and Heritage Island are now referred to Kingman (K); likewise, all plots in the A and B fringe wetlands are subsequently named Fringe A (A).

Methods

Treatments in Kingman and the Anacostia River Fringe study areas are replicated in their treatment and sampling layout in each of the identified *Phragmites* cells / polygons (Figure 1). These treatments are organized into mowed and un-mowed cells, with Habitat and Rodeo treatments under each of these conditions. Additionally, there are mowed and un-mowed Habitat/Rodeo mix treatments, which according to Invasive Plant Control, Inc. (IPC, personal communication) are often applied to increase effectiveness. Two control cells did not receive herbicide application in order to determine natural change in the

Phragmites in comparison to the chemically sprayed plots. One of the control cells was mowed in order to determine the influence of solely mowing.
Herbicide application concentrations and rates are as follows:

- Habitat (1% volume/volume imazapyr at 6 pints per acre)
- Rodeo (1.5% volume/volume glyphosate at 7.5 pints per acre)
- Habitat/Rodeo mixed (3% volume/volume Rodeo (glyphosate) & 5 pints per acre Habitat (imazapyr).

Each *Phragmites* polygon has its own complete treatment aside from the central long 0.29 acres polygon next to Heritage Island, which was split and transects separated enough to not have translocation and mixing of each herbicide affecting adjacent plots, removing a potential issue (Figure 1).

Treatment Options by Each Area (Acre) & Location

	Habitat (acre)	Rodeo (acre)	Rodeo & Habitat (acre)	Control (acre)
Mowed	0.29 (K) / 0.24(A)	0.29(K) / 0.16(A)	0.23(K)	0.09(A)
Un-mowed	0.06(K) / 0.19(A)	0.11(K) / 0.22(A)	0.12(A)	0.05(K)

Herbicide application was conducted from the ground using a low-volume backpack sprayer and a low-volume tank with a hose reel from an ATV or RTV completed by Invasive Plant Control Inc. Applications were conducted by a certified applicator in accordance with label specifications and all applicable local, state and federal regulations. The herbicide was applied October 22nd, 2013.

Mechanical mowing occurred in July-September prior to herbicide application, with the exception of the Kingman control, which took place on November 11th 2013 (Figure 2).

Methods for Experimental Monitoring Plan

The monitoring methods followed those laid out in the USGS and DDOE Kingman and River Fringe monitoring efforts (Krafft *et al.* 2009; 2010). The treatments, consisting of each herbicide and mowing regime are uniform, randomly covering an equal and diverse area within each of the separate *Phragmites* cells, or polygons (Figure 1). Mowed vs. un-mowed areas and the different herbicide treatments are combined in their respective cells. The attached Figure 1 outlines the various application treatment areas of each herbicide separately and combined. The combined application of both herbicides may have an additive effect and better treatment results. Additionally, mowed vs. un-mowed areas are highlighted and control plots where no herbicide will be applied are identified. Transect lines are laid out in an East to West orientation and show their general location in Figure 1. Transect lines on the figure are scaled to size.

Transects

The table below details a total of 36 transects between the various treatment options, three transects per treatment, laid East to West perpendicular to the shoreline through each *Phragmites* polygon (Figure

1). Kingman experimental area transects are 15 m in length, with three transects spaced evenly through each target treatment cell among the Heritage Island Wetland (6 transects) and Kingman Marsh Area 2 (12 transects). Anacostia Fringe Area transects are 25 m in length, with three transects spaced evenly within each target polygon for a total of 18 transects, including the 3 transects in the Anacostia Fringe Area *Phragmites* polygon below East Capitol Street.

Number of Transects per Treatment and Experimental Area

Treatment Option	Kingman Experimental Areas /			Anacostia Fringe Experimental Areas		
	Polygon Treatment Code	Transect #	Plot #	Polygon Treatment Code	Transect #	Plot #
Control	K-C-M	1, 2, 3	1 to 4	A-C-U	1, 2, 3	1 to 4
Habitat Mowed	K-H-M	1, 2, 3	1 to 4	A-H-M	1, 2, 3	1 to 4
Habitat Un-mowed	K-H-U	1, 2, 3	1 to 4	A-H-U	1, 2, 3	1 to 4
Rodeo / Habitat Mowed	K-RH-M	1, 2, 3	1 to 4			
Rodeo / Habitat Un-mowed				A-RH-U	1, 2, 3	1 to 4
Rodeo Mowed	K-R-M	1, 2, 3	1 to 4	A-R-M	1, 2, 3	1 to 4
Rodeo Un-mowed	K-R-U	1, 2, 3	1 to 4	A-R-U	1, 2, 3	1 to 4

Kingman and Fringe treatments both have a small control plot of *Phragmites* in adjacent areas which will not have any chemical treatments to ensure that a mass *Phragmites* die off did not occur in the area during the study to skew the results. The two control polygons were split between mowed and unmowed, with the Fringe A control being left un-mowed and Kingman receiving the physical mowing. After the second year these plots can be targeted with the most effective treatment determined from the study.

Sample Plots

Four 1m x 2m permanent sampling plots were randomly placed along each of 36 transects (144 plots). One long (3m above marsh surface) and one short (1m above marsh surface) segments of PVC pipe were placed into the marsh surface at the corners of each plot which are oriented with the 2m side of the plot perpendicular to the transect line. At least one meter separated each sample plot in order to increase sampling uniformity among the area and again a one meter buffer at the edge of the extent of *Phragmites* within each transect. If a random coordinate did not meet the plot location criteria, a new random coordinate was chosen and plot identified.

Data Collection

Upon the establishment of marsh transect lines photos document each line from the start (landside) of each transect. Photos were retaken at each mowed transect after mowing and all transects were photographed again the following year when collecting numerical data.

Data on percent cover of live *Phragmites*, within each plot, before and after control efforts were made through ocular estimates. All non-*Phragmites* species were identified and their percent relative cover determined as a whole before and after *Phragmites* management. Vegetative surveys (ocular estimates of percent coverage) of all plots along transect lines were taken after mowing and prior to herbicide applications. The plot surveys were repeated again the second year (Figure 2, 3).

Statistical Analysis

Due to non-normality issues, statistical analysis was completed through a logistic regression indicating the differences in site (Anacostia Fringe and Kingman), herbicide treatment (Habitat, Rodeo, Habitat/Rodeo and control), and mowing (mowed and un-mowed). A species list and ocular evaluations of percent cover of non-*Phragmites* species throughout the sample plots were also determined. Percent change in non-*Phragmites* species cover was analyzed using an analysis of variance, ANOVA.

Results

Phragmites Data

Results were analyzed using the Statgraphics program (Centurion XVI, Version: 16.2.04). As a result of non-normality within the composed data, presumably a consequence of using a percentage-based collection system, the data could not be analyzed in its current state (Figure 4). The ocular estimates based on percent *Phragmites* from pre-and-post control were modified into a binary system, where treatments that possessed a change in *Phragmites* greater than or equal to (≥) 75% received a value of ‘1’, while any plot that had a change less than 75% received a ‘0’. This conversion allows the non-normal data to be successfully analyzed through a logistic regression, indicating the significance between the three independent variables (site, herbicide type, and mow treatment) without violating any statistical assumptions. A logistic regression, in essence, attempts to mathematically model the outcome of the dependent value (0 or 1) through the influencing independent, X factors. In other words, the X variables are used to create an equation, which predicts the probability, under specific circumstances, that the dependent variable receives a 1: defined as a 75% or greater reduction in *Phragmites*. A reduction of 75% or greater was selected as ‘success’ because an ecosystem can “benefit from interspersion of *Phragmites* with other plant species and water… *Phragmites* should be controlled only to the degree necessary to achieve management objectives” (Cross 1989). In this case, monocultures of *Phragmites* were the target of control; thus, a 75% or greater reduction is sufficient for success.

Non Phragmites Data

Due to the fact that the non-*Phragmites* data followed a normal curve, ANOVA was used to analyze the variance between the dependent factors (herbicide, mow treatment and site). The Statgraphics program (Centurion XVI, Version: 16.2.04) was used for all non-*Phragmites* data investigation.

Phragmites results

<u>Logistic Regression - Binary Change in *Phragmites*</u>

Dependent variable: Change in *Phragmites* (75% or more)

Factors:

Habitat (None compared to Habitat)
Rodeo (None compared to Rodeo)
Rodeo-Habitat (None compared to Rodeo-Habitat Mix)
Mowed (Mowed compared Unmowed)
Site (Fringe A/B compared to Kingman)

Estimated Regression Model (Maximum Likelihood)

		Standard	*Estimated*
Parameter	*Estimate*	*Error*	*Odds Ratio*
CONSTANT	-4.91006	0.882844	
Habitat	0.909777	0.785096	2.48377
Rodeo	2.52823	0.793694	12.5313
Rodeo-Habitat	3.512	0.885777	33.5153
Mowed	3.64195	0.646642	38.1663
Site	1.44824	0.588799	4.25562

Table 1: The output shows the results of fitting a logistic regression model to describe the relationship between change in *Phragmites* and 5 independent variable(s). The equation of the fitted model is:

Change in *Phragmites* = exp(eta)/(1+exp(eta))

Where:

eta = -4.91006 + 0.909777*Habitat + 2.52823*Rodeo + 3.512*Rodeo-Habitat + 3.64195*Mowed + 1.44824*site

Analysis of Deviance

Source	*Deviance*	*Df*	*P-Value*
Model	77.7902	5	0.0000
Residual	117.817	138	0.8924
Total (corr.)	195.608	143	

Table 2: Because the P-value for the model in the Analysis of Deviance table is less than 0.05, there is a statistically significant relationship between the variables at the 95.0% confidence level. In addition, the P-value for the residuals is greater than or equal to 0.05, indicating that the model is not significantly worse than the best possible model for this data at the 95.0% or higher confidence level.

Percentage of deviance explained by model = 39.7685
Adjusted percentage = 33.6337

-Shows that the percentage of deviance in change in *Phragmites* explained by the model equals 39.7685%. This statistic is similar to the usual R-Squared statistic. The adjusted percentage, which is more suitable for comparing models with different numbers of independent variables, is 33.6337%.

Likelihood Ratio Tests

Factor	*Chi-Square*	*Df*	*P-Value*
Habitat	1.4332	1	0.2312
Rodeo	12.9432	1	0.0003
Rodeo+Habitat	21.8669	1	0.0000
Mowed	55.1912	1	0.0000
Site	7.11351	1	0.0076

Table 3: In determining whether the model can be simplified, notice that the highest P-value for the likelihood ratio tests is 0.2312, belonging to Habitat. Because the P-value is greater or equal to 0.05, that term is not statistically significant at the 95.0% or higher confidence level. This signifies that the Habitat herbicide had little to no influence on the ‘success’ (reaching 75% or higher) rate of removing *Phragmites*.

Coefficents:	Estimate	Std. Error	z-value	Pr (>|z|)
Intercept	-1.3050	0.6477	-2.015	0.04592*
Rodeo vs. No Herbicide	2.4427	0.7780	3.140	0.00169**
Rodeo vs. Habitat-Rodeo	-0.1708	0.9885	-0.86279	0.86279
Significant Codes	0 = ‘***’	0.001 = ‘**’	0.01 = ‘*’	

Table 4: A separate statistical analysis was completed in R in order to demonstrate an interaction between Rodeo and the Rodeo-Habitat mix; highlighting the increased efficiency in the mix cannot be statistically separated from Rodeo alone.

Non-Phragmites Results

Analysis of Variance for % Change in Non-*Phragmites* Cover - Type III Sums of Squares

Source	*Sum of Squares*	*Df*	*Mean Square*	*F-Ratio*	*P-Value*
MAIN EFFECTS					
A:Herbicide	16140.0	3	5379.99	5.50	0.0013
B:Mowed	2213.9	1	2213.9	2.26	0.1347
C:Site	252.188	1	252.188	0.26	0.6124
RESIDUAL	134940.	138	977.824		
TOTAL (CORRECTED)	153294.	143			

All F-ratios are based on the residual mean square error.

Table 5: The ANOVA table decomposes the variability of % change in non-*Phragmites* cover into contributions due to various factors. Since Type III sums of squares (the default) have been chosen, the contribution of each factor is measured having removed the effects of all other factors. The P-values test the statistical significance of each of the factors. Since one P-value is less than 0.05, this factor has a statistically significant effect on % change in non-*Phragmites* cover at the 95.0% confidence level.

Multiple Range Tests for % Change in Non-*Phragmites* Cover by Herbicide

Method: 95.0 percent LSD

Herbicide	*Count*	*LS Mean*	*LS Sigma*	*Homogeneous Groups*
Habitat	48	11.5104	4.51346	X
Rodeo	48	18.6875	4.51346	X
None	24	20.375	6.383	X
Rodeo/Habitat	24	42.9583	6.383	X

Contrast	*Sig.*	*Difference*	*+/- Limits*
None – Habitat		8.86458	15.4577
None – Rodeo		1.6875	15.4577
None - Rodeo/Habitat	*	-22.5833	17.849
Habitat - Rodeo		-7.17708	12.6211
Habitat - Rodeo/Habitat	*	-31.4479	15.4577
Rodeo - Rodeo/Habitat	*	-24.2708	15.4577

* denotes a statistically significant difference.

Table 6: This table applies a multiple comparison procedure to determine which means are significantly different from which others. The bottom half of the output shows the estimated difference between each pair of means. An asterisk has been placed next to 3 pairs, indicating that these pairs show statistically significant differences at the 95.0% confidence level. At the top of the page, 2 homogenous groups are identified using columns of X's. Within each column, the levels containing X's form a group of means within which there are no statistically significant differences. The method currently being used to discriminate among the means is Fisher's least significant difference (LSD) procedure. With this method, there is a 5.0% risk of calling each pair of means significantly different when the actual difference equals 0.

Discussion

Herbicide

Habitat's active herbicidal chemical is imazapyr at 27.77% volume. Imazaypr is a non-selective herbicide, used for the control of a broad range of plant life, which interferes with the enzyme acetohydroxy acid synthase causing a halt in the production of three crucial amino acids for plant survival: valine, leucine, and isoleucine. These amino acids are necessary for protein synthesis and cell growth (Tu, Hurd, and Randall 2001). The persistent, slow metabolizing, herbicide can be absorbed through above ground plant biomass, as well as through root systems, where it is translocated to the meristematic tissues producing the inhibition of enzymatic function (Tu, Hurd, and Randall 2001). Typically, plant mortality is a slow process taking many weeks due to stored amounts of valine, leucine, and isoleucine.

Environmentally, imazapyr possesses a low toxicity because invertebrates and vertebrates do not actively produce these inhibited amino acids; therefore, the herbicide does not have the potential to interfere with the metabolic processes of fauna. Persistence and mobility of the herbicide is greatly influenced by the environmental pH. In acidic ecosystems (pH < 5), imazapyr is adsorbed to soil particles, decreasing movement and plant uptake, where it is degraded primarily through microbial interaction within one to seven months (Mangels 1991). In a characteristic marsh setting, the pH is generally neutral, causing imazapyr to remain mobile and available for uptake, where it is rapidly photodegraded, a half-life of two days, in an aquatic settings (Marshes, Mallipudi 1991, Mangels 1991). Imazapyr in an aquatic setting is of little concern due to its high rate of degradation through ultraviolet sunlight.

Rodeo contains glyphosate, 58.8% by volume, which serves as the plant mortality causing agent. Glyphosate, similar to imazaypr, is a non-selective systemic herbicide, which inhibits a different enzyme, 5-enolpyruvylshikimic acid-3-phosphate synthase, leading to a loss in the production of tyrosine, tryptophan, and phenylalanine. These aromatic amino acids are critical for the production of metabolic proteins, through their inhibition, the plant can no longer survive (Carlisle & Trevors 1988). Glyphosate is absorbed by plants primarily through direct above ground biomass contact, and is not actively taken up through the root systems (Hance 1976).

Glyphosate can remain present in the environment for a considerable time, with a half-life from several weeks to years (Tu, Hurd, and Randall 2001). This is the result of glyphosate's strong adsorption to soil particles, which limit its mobility and increase environmental residence periods (Tu, Hurd, and Randall 2001). Glyphosate is readily soluble; however, due to its extreme adsorption rate to the soil, the herbicide does not remain free in an aqueous setting for long periods of time. Water contamination issues only occur when glyphosate-attached soil particles wash down stream. (Sprankle 1975). Microbial degradation is the main pathway of removal, although this can prove to take considerable time as a consequence of soil adsorption, depending on soil types, which limits microbial interaction and makes root uptake virtually non-existent (Sprankle 1975). Glyphosate has a low toxicity to vertebrates and invertebrates due to its 'mode of action', which occurs in the chloroplasts of most plant species as well as the fact that once in contact with soil, mobility is insignificant.

Forty-eight plots were treated with Rodeo, forty-eight with Habitat, and twenty-four received no herbicide, data collection occurred in the fall of 2013 then post-control again in the summer of 2014 (figure 3). Based on the collected data, these two herbicides, imazapyr and glyphosate, performed at significantly different levels when attempting to remove *Phragmites* populations within the Anacostia River. Rodeo and Habitat were individually compared to a control *Phragmites* population and monitored for 'success' (a 75% or more removal), under varying site conditions (Kingman/Heritage island and Fringe A/B wetlands), as well as different mowing treatments (mowed and unmowed) from 2013 to 2014 with control measures occurring in the fall of 2013. Rodeo consistently removed more *Phragmites* from the populations than the Habitat herbicide, with respective p-values, in relation to no herbicide, of: 0.003 and 0.2312. With a p-value < 0.05, we can conclude with a 95% confidence level that Rodeo reaches 'success' (a removal of ≥75% of *Phragmites* from a population) more than no herbicide treatment. Conversely, Habitat, with a p-value > 0.05 indicates there is no statistical evidence at the 95% confidence level that Habitat is not equal to no herbicide treatments. In other terminology: Rodeo ≠ no herbicide, while Habitat = no herbicide. Figures 5 and 6 highlight this discrepancy.

When regulating *Phragmites* through chemical control methods, systemic herbicides, which translocate throughout the plant causing above and below ground mortality, are considered the most effective (Cross 1989). Both imazapyr and glyphosate are systemic herbicides, with imazapyr, more expensive than glyphosate, generally noted as being more successful (Treatment). However, this notion is challenged based on the data collected. Glyphosate preformed at a significantly higher rate than imazapyr for reasons that can be hypothesized, but not confirmed without further research. The *Phragmites* populations targeted were located within tidal emergent wetlands with a neutral pH and inundation occurring twice per day. Glyphosate adsorbing to the soil particles may have possessed a longer term of mortality, as opposed to imazapyr, which has little adsorption capabilities, and degrades readily in an aquatic settings.

Lastly, an herbicide mix of Rodeo and Habitat was applied to specific *Phragmites* populations, producing the greatest determined ‘successes’ (Figure 7). The mix was evaluated to be the most statistically significant herbicide factor when compared to a no herbicide mix with a p-value of 0.000. This suggests that the Rodeo Habitat mix was the most effective chemical control of *Phragmites*. Yet, upon further statistical analysis, the Rodeo-Habitat mix cannot be considered significantly different from the Rodeo application alone. Thus, although both Rodeo and the mix are significant from a no herbicide treatment, the discrepancies between the two treatments (glyphosate and mix) cannot be accounted for statistically (Table 4). Further research is needed to determine if the mix of imazapyr and glyphosate is more successful as a chemical control than simply glyphosate.

Mowing

Mowing *Phragmites* with the aim of eradication is a complex matter; the effectiveness of mowing a *Phragmites* population is heavily related to the timing of the mow. Mowing *Phragmites* stands in the early growing season can promote growth and increase population density (Treatment). Conversely, cutting in the fall season, before the stalks have begun to send energy storages into the root systems can reduce energy reserves and population abundance (Treatment, Granholm 1994). Often times a mechanical removal of *Phragmites* is used in conjunction with a chemical treatment to increase efficiency. This study aims to assess the effect of mowing prior to an herbicide treatment.

Shown in figure 2, mowing of specific populations occurred in the fall of 2013 prior to the application of the different herbicides, and ocular estimates of the change in *Phragmites* were assessed to determine the effectiveness of mowing or not mowing (unmowed) on the determined ‘success’ of *Phragmites* removal. The different mowed and unmowed treatments were statistically significant with the p-value of 0.000, presented in table 3, suggesting that the presence of mowing had an extensive effect on the outcome of achieving ‘success’. Mowing prior to an herbicide treatment greatly reduced the viability of the herbicide upon the *Phragmites*. As portrayed in figure 8, mowing a site promotes very little opportunity to reach success, or a ≥75% removal. Compared to the 35% chance (excludes herbicidal effects) of success associated with leaving a site unmowed. In congruence with much of the literature, mowing prior to an herbicide treatment (with the exception of immediate, direct herbicide treatment) is a hindrance to the removal process. With less plant surface area for Rodeo and Habitat to interact with, mowing could reduce the plant uptake of the applied chemical, limiting mortality (Ailstock, Bushman, and Norman 2001). Secondly, mowing may increase the resurgence of *Phragmites* through increase availability of resources, sunlight (Cross 1989). Mowing should be performed roughly two weeks after the initial herbicide, to allow sufficient translocation of the systemic herbicide; yet, this is disputed, and some data suggest mowing after herbicide application is nominal (Granholm 1994, Derr 2008).

Site

Site differences can play a role in the success of *Phragmites* removal, typically in the form of inundation levels as related to substrate elevation. Although *Phragmites* is tolerant to fresh water, making inundation a futile control for existing stands, frequent flooding, as is representative in a tidal setting, may suppress the spread of new seedlings (Outreach Center). This is especially applicable to those sites which received mowed treatments causing inundation to influence the regeneration of *Phragmites*; thus, in the low-marsh Kingman site, we see a decrease in *Phragmites* following only a mow treatment (figure 9, KCM) (Outreach Center).

Based on the elevation of the Phragmites stands and tidal fluctuations in the Anacostia River, inundation levels may possess some influence on the success of removal. The data reveal that site variations, between a low-marsh where inundation occurs regularly (K) to a highland marsh where flooding is not as prevalent (A), possess statistical significance with a p-value of 0.0076. Figure 10 symbolizes the differences between the low and high marshes selected, signifying that the low-land marsh reached ‘success’ more frequently when compared to the upland marshes. There are several factors between the sites that could have had an impact on the effectiveness of the *Phragmites* removal between Kingman and Fringe A/B; however, inundation may have played a key role in the determined ‘successes’. More research is needed to attribute these site variations to solely inundation, or to another influencing factor.

Non-Phragmites Resurgence

Phragmites stands are detrimental to ecosystems because of their monotypic nature. The plant has the ability to suppress native vegetation, alter existing hydrology, and displace native wildlife within the region (Ludwig 2003). Therefore, a successful removal of the wetland plant is necessary; nevertheless, the restoration process is not complete without a successful resurgence of the native plant communities. Native plants are integral to the wetland restoration process, providing the foundation for insects and wildlife, which heavily rely on the natural environment they inhabit. “Native plants have evolved with native wildlife" (Tallamy 2007). Fauna communities have developed to utilize native plants from the shape of flowers to the chemical content of leaves; native plants are tailored to the native wildlife of the area (Tallamy 2007). Thus, the resurgence of native plants is a critical component to an accomplished *Phragmites* removal.

The scope of this study included an ocular measurement (%) of the native non-*Phragmites* species that arose as a result of *Phragmites* mortality. Table 5 demonstrates through the lens of herbicide applications, mowing treatments, and site differences, which dependent factors yielded a significant influence upon the resurgence of non-*Phragmites* species. Herbicide, containing a p-value of 0.0013, was the only statistically significant factor, with an emphasis on the Rodeo/Habitat mix treatment exhibiting the statistical difference, determined in Table 6. Figure 11 illustrates these variances between herbicidal treatments. The noted difference in non-*Phragmites* species recovery could be an indicator of the increase efficiency of *Phragmites* removal through the mixed herbicide application. Figure 12 depicts the mowed treatments as possessing a slightly higher recolonization of species, likely due to increased availability of resources with more sunlight penetration; yet, this difference in not statistically supported. Site variations in low and high marshes indicate higher marshes possessed a greater resurgence of non-*Phragmites* species: likely due to more species diversity in higher regions and factors that cannot be explained through elevation, such as, openness, vulnerability to goose grazing, and seed sources. Likewise, this difference is not statistically significant (Figure 13).

Conclusions

The objective of this study was to successfully determine which applied *Phragmites* removal treatments are the most successful in the tidal Anacostia River, and riverine wetlands. The synthesized data aim to shape decision making, increase efficiency, and improve success when attempting to perform an invasive *Phragmites* management operation. The Anacostia Watershed Society will use this information to progress future *Phragmites* reductions (control and containment) and implement further research opportunities.

Collected data, in the 2013-2014 period, from the 144 plots dispersed throughout different regions on the Fringe A/B and Kingman/Heritage Island in the Anacostia River (Figure 1), following a management technique, indicate there are significant statistical differences between control practices. Site considerations, mowing application, and herbicide treatment were taken into account and assessed for *Phragmites* removal 'success', defined as a ≥75% reduction. Results suggest all dependent variables (site, mow, and herbicide) possessed a significant influence on the observed success.

The low (K) marshes out preformed the high (A) in *Phragmites* removal, potentially due to inundation differences, with the low areas receiving more water, limiting *Phragmites* regrowth (Carlson, Kowalski, and Wilcox 2009). Inundation can be a useful prediction tool when attempting to determine the level of action needed to removal a *Phragmites* monoculture. Resurgence of non-*Phragmites* species data show a slightly higher revegetation within the high marshes, which could be due to a larger seed bank; however this is not statistically significant. Further research into the site specifics is needed before any supported claims can be produced about the variations in site.

Mowing, prior to herbicide application (spraying), in comparison to not mowing (unmowed) sites were calculated as statistically significant. Those plots where mowing did not occur had a markedly higher rate of ‘success’. This is critical information for a spray-herbicide treatment because it implies that mowing a region generates a situation where herbicide is not readily absorbed into the plant tissue and translocated throughout the plant, as opposed to an unmowed stand where herbicide uptake is more prevalent (Ailstock, Bushman, and Norman 2001). More research is needed to conclude whether mowing should occur after an herbicide application; nevertheless, research suggests that mowing two weeks after application could have a positive effect on removal. Non-*Phragmites* species recovery was insignificant under different mowing applications; yet, other sources suggest this is not necessarily the case.

Lastly, herbicide type, imazapyr and glyphosate, were assessed for efficiency of removal. The data illustrate that glyphosate removed *Phragmites* stands, ≥75%, at a higher rate when compared to imazapyr. Rodeo out-performed Habitat applications significantly, making this data of importance to future removal operations, highlighting that the less-expensive Rodeo should be selected for an improved *Phragmites* removal. A Rodeo/Habitat mix treatment was also applied, and contains the largest statistical difference, suggesting the mix manufactured the greatest probability of success; however, upon further analysis, the data could not show statistical significance from the Rodeo alone. In other words, more research is required to determine if the increased efficiency of the *Phragmites* removal from the mix can be attributed to the sole influence of Rodeo. Non-*Phragmites* species cover was statistically significant in the Rodeo/Habitat mix, but showed no variation between the three other treatments (no herbicide, Habitat alone, and Rodeo alone), highlighting the benefits of the Rodeo/Habitat mix on marsh revegetation following a chemical control. Again, a more comprehensive experiment must be completed to determine the particulars influencing this discrepancy.

The Anacostia Watershed Society recommends that monitoring and further, more specific, examinations of *Phragmites* removal be completed in order to identify the particular mechanisms associated with the most successful *Phragmites* deductions.

Acknowledgments

Many individuals were of great importance to the successful completion of the *Phragmites* management study in the Anacostia River. AWS would like to denote a special thanks for collaboration and partnership to: Peter May, of Biohabitats; Cairn Krafft, with the U.S. Geological Survey; Dick Hammerschlag; Damien Ossi, from the District Department of the

Environment and D.C. Cooperative Weed Management Area; Jeff Hatfield, of the U.S. Geological Survey; Mark Frey, from the National Park Service: Exotic Plant Management Team; Mikaila Milton, with the National Park Service: National Capital Parks East; Dennis Chestnut, of Groundwork Anacostia; and Erin McMahon, masters candidate at University of Columbia.

Figures

Figure 1: Shows a map of *Phragmites* populations in the Anacostia River and their received treatments.

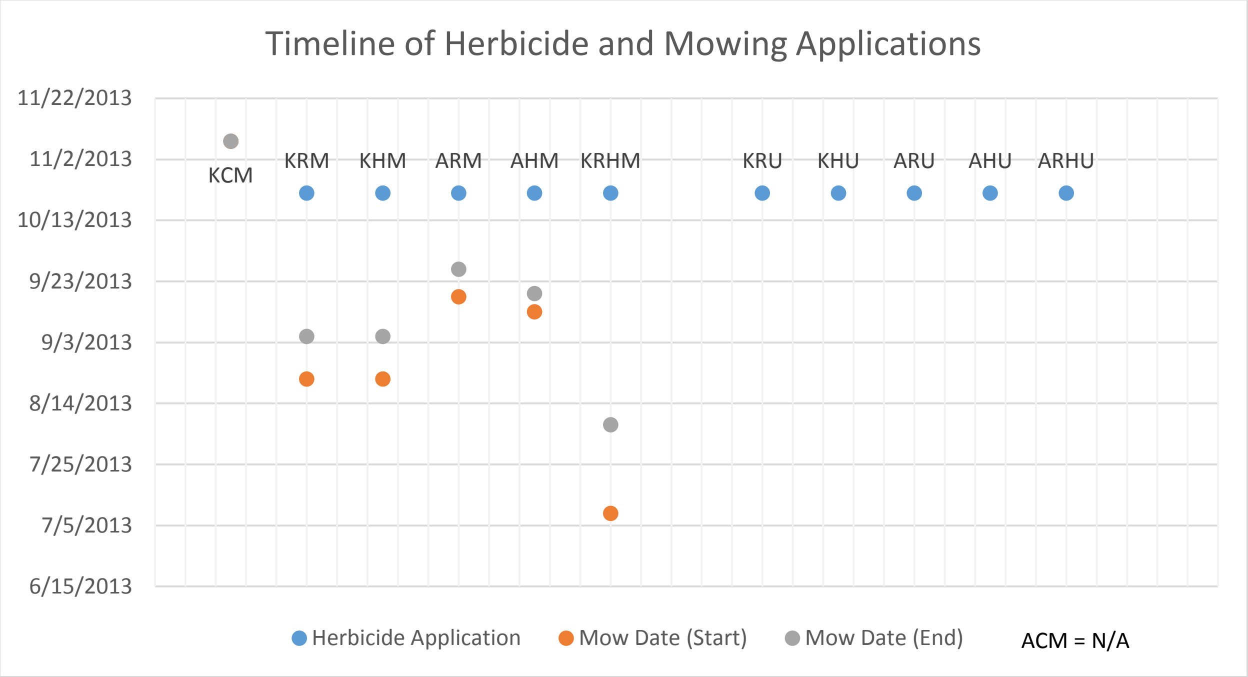


Figure 2: Depicts the dates of herbicide and mowing controls for each treatment. *Mowing was completed on the same day for KCM.

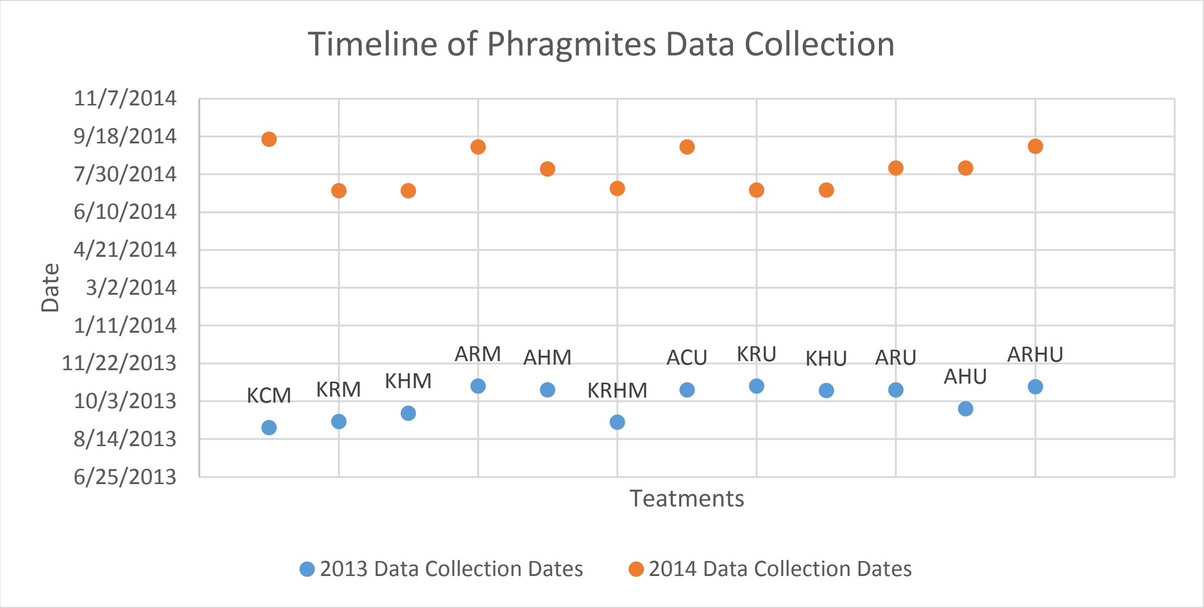
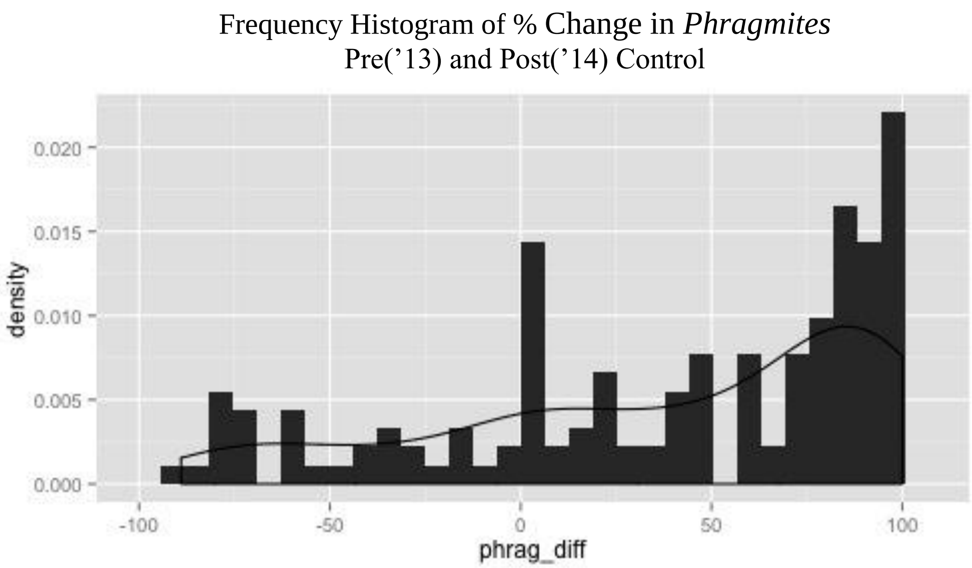
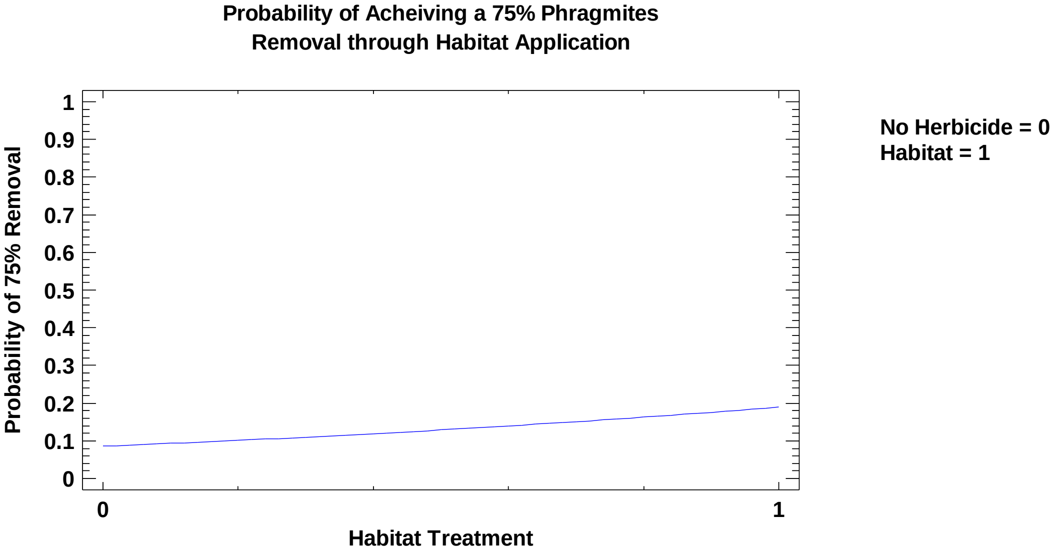


Figure 3: Shows the data collection dates for each individual treatment from pre-control 2013 to post control 2014.



Frequency Histogram of % Change in *Phragmites*
Pre('13) and Post('14) Control

Figure 4: Indicates the non-normal distribution of the data, skewed to the left.



Probability of Acheiving a 75% Phragmites Removal through Habitat Application

No Herbicide = 0
Habitat = 1

Figure 5: This graphical representation of the applied Habitat herbicide, to the selected *Phragmites* regions in the Anacostia River, depicts the percent difference in 'success' (a 75% removal of *Phragmites*) between no herbicide (0) and Habitat (1). As shown, *Phragmites* under a no herbicide treatment has a ~9% likelihood of reaching 75% removal rate while under an applied Habitat condition the value raises to ~20%. However, the difference between these values is not statistically supported.

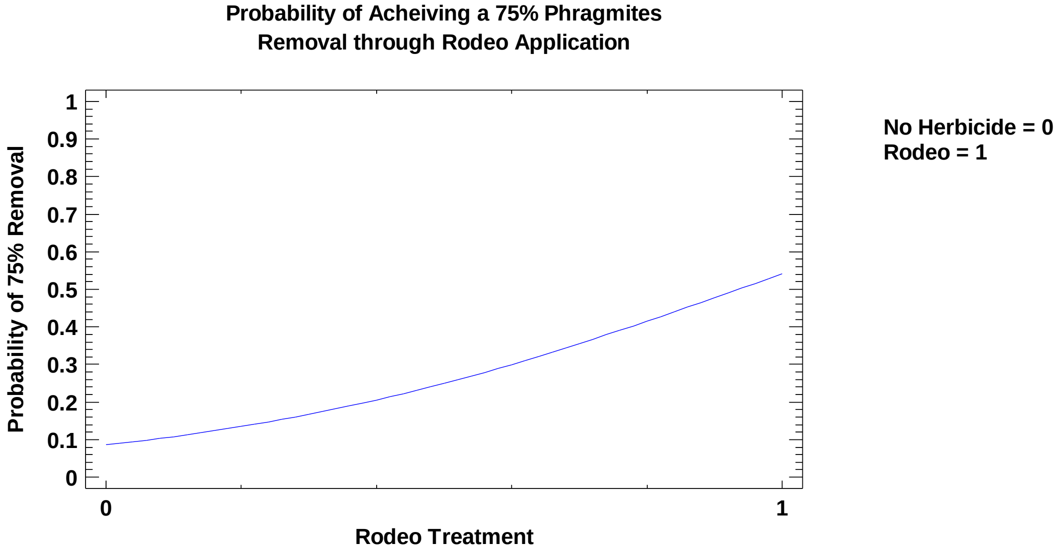


Figure 6: This graph portrays the difference in probability of a 75% removal of *Phragmites* under a no herbicide condition (0) and an applied Rodeo herbicide (1). Under a no herbicide treatment, the base rate probability of reaching 'success' (75% removal or greater) is ~9%; in contrast, the probability of removing 75% or more *Phragmites* in a region after the application of Rodeo is raised to ~55% of reaching 75% removal rate. These difference between no herbicide and Rodeo is statistically significant.

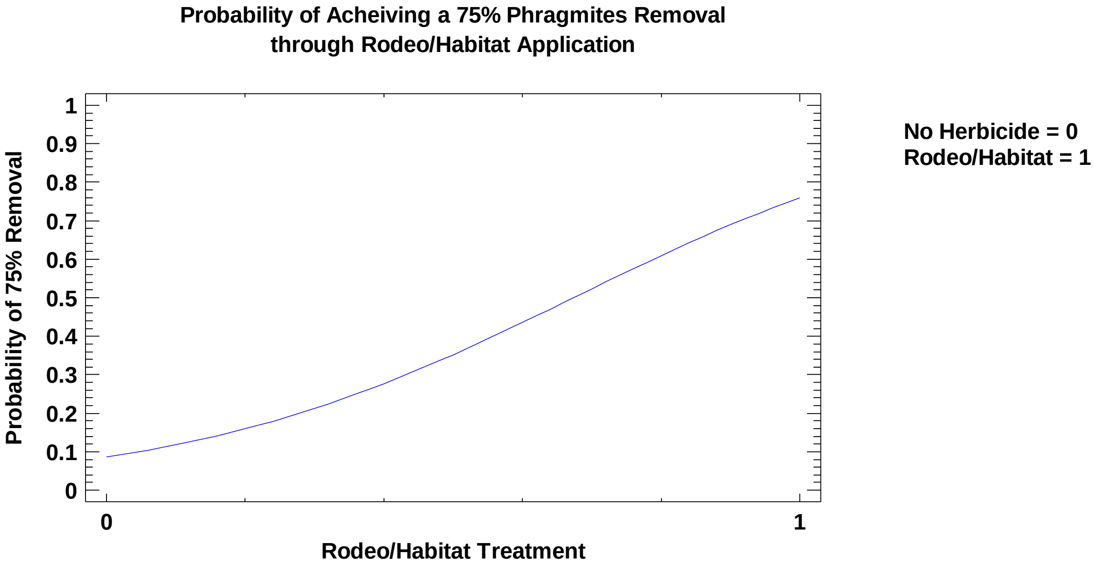
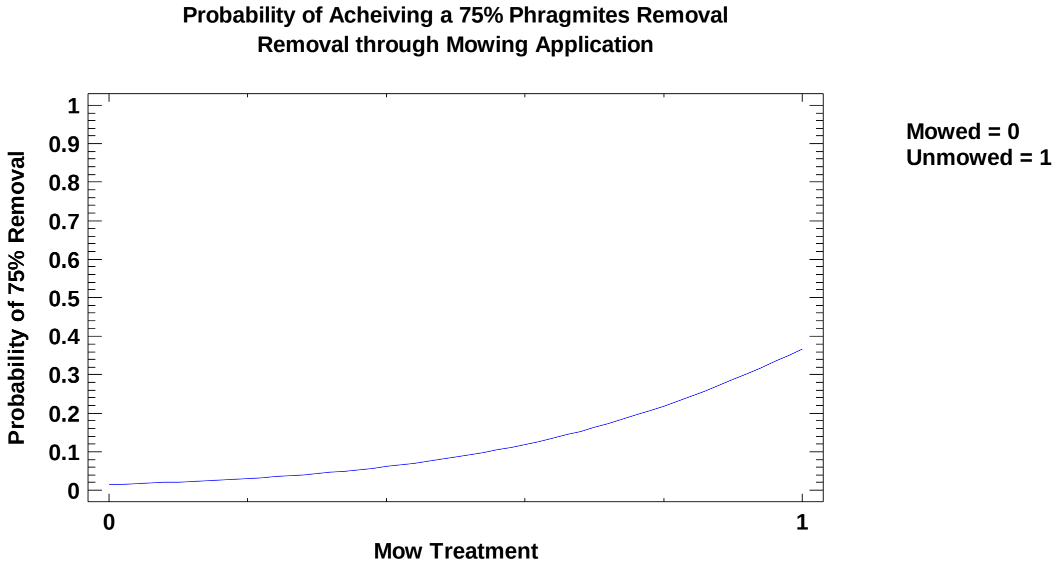
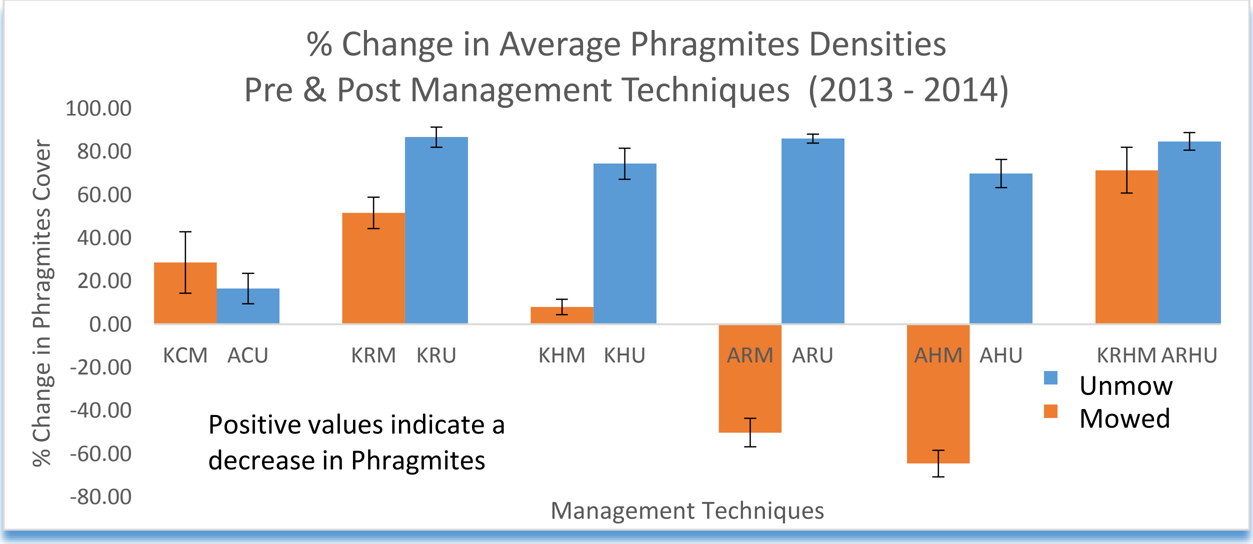


Figure 7: The graph illustrates the difference in *Phragmites* removal between treatments that received no herbicide (0), and *Phragmites* populations that were sprayed with a mixture of a Rodeo/Habitat herbicide (1). Treatments receiving no herbicide possess a probability of removing 75% or more *Phragmites* of 9%; whereas, the application of the Rodeo/Habitat mix causes this value to increase greatly, suggesting that there is a 75% probability that 75% or more *Phragmites* in a population will be removed after the application of the Rodeo/Habitat mix herbicide. These values produced are statistically significant.



Probability of Acheiving a 75% Phragmites Removal
Removal through Mowing Application

Figure 8: This diagram highlights the difference between mowed and ‘unmowed’ treatments. Indicating that under a mowed (0) setting (mowing occurred prior to herbicide application) there is a significantly less probability of achieving a 75% or greater eradication of *Phragmites* with a base rate of ~0%. Conversely, under an unmowed condition, prior to herbicide application, the chances of obtaining a 3/4ths removal of *Phragmites* significantly increases to ~35%. Mowing versus not mowing is statistically different.



% Change in Average Phragmites Densities
Pre & Post Management Techniques (2013 - 2014)

Positive values indicate a decrease in Phragmites

Figure 9: Shows the average change in *Phragmites* from 2013 (pre-treatment) to 2014 (post treatment) per plot. A positive value represents a general decrease in overall *Phragmites*.

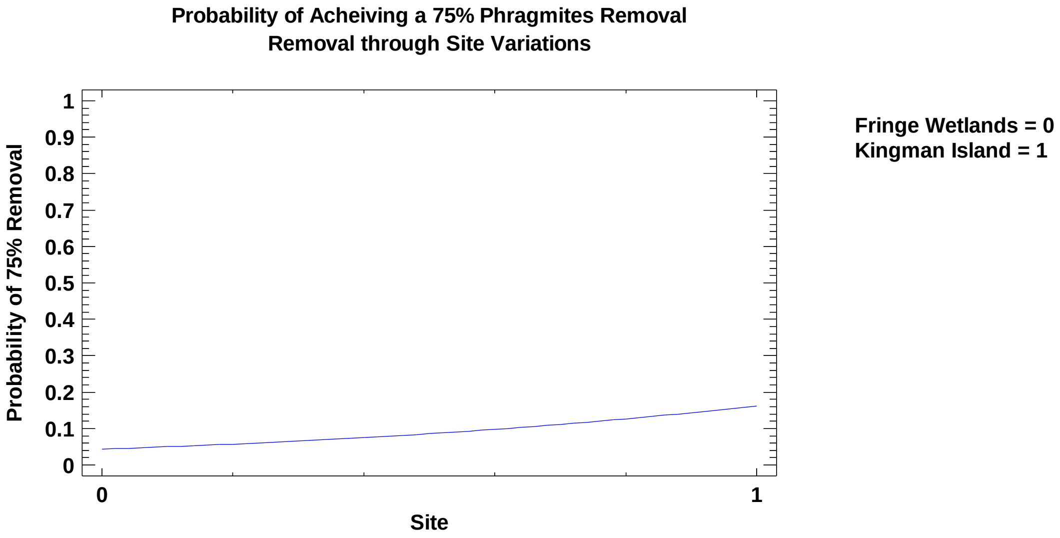


Figure 10: This graph represents the variations in site that produced a statistically significant difference between the low-land marshes, Kingman, as opposed to the upland fringe wetlands. The fringe wetlands were notably less successful at removing 75% or more *Phragmites* from the identified populations, containing a ~5% removal rate; while, the Kingman Marshes removed 75% or more *Phragmites* following the treatments with a probability of ~15%. Meaning that Kingman Island contains a probability roughly three times greater that the fringe wetlands when removing 3/4ths or more *Phragmites* coverage from a population.

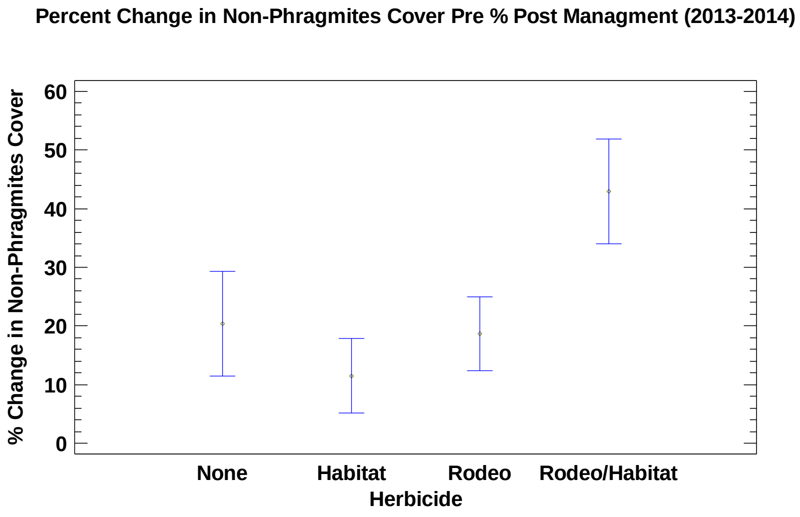


Figure 11: This graph indicates the percent change in non-*Phragmites* cover as related to herbicide application only. There is statistical significance ($p < 0.05$) in the herbicide applications influence on the resurgence of non-*Phragmites* species within the treatments sites in the Anacostia River. However, this graph depicts that the statistical significance comes from the Rodeo/Habitat mix in comparison to the other three treatments (no herbicide, Habitat, and Rodeo).

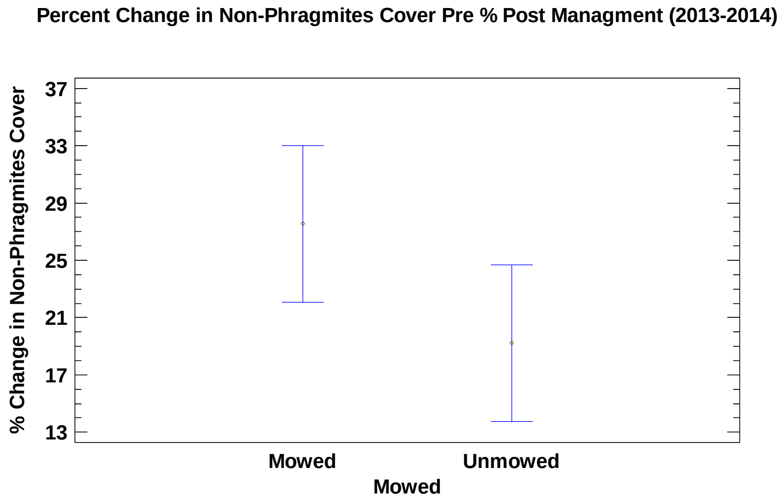


Figure 12: This plot highlights the marked difference between the mowed sites and the unmowed sites in relation to the reestablishment of non-*Phragmites* species. As shown, a mowed site produced a higher re-vegetation; yet, this difference from the unmowed sites is not statistically supported.

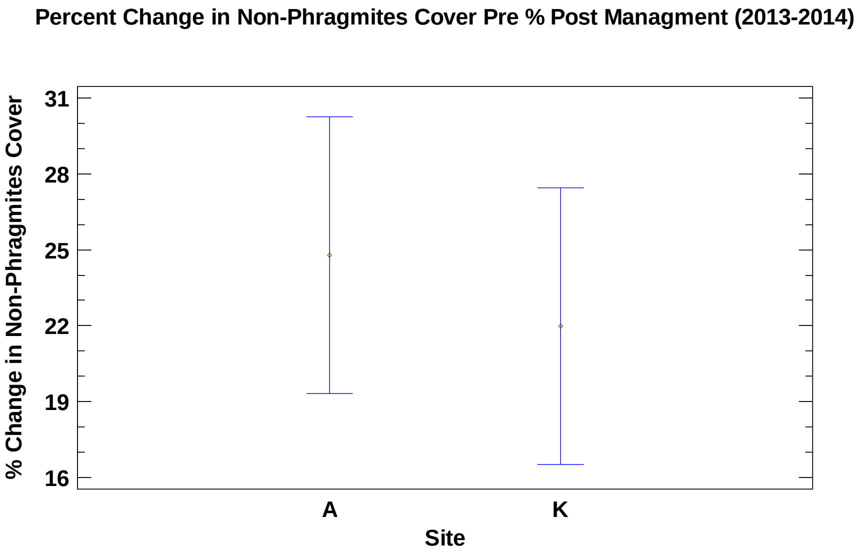


Figure 13: Shows the variation in non-*Phragmites* re-vegetation, following a control attempt of *Phragmites*, between a low-land marsh (K) and an upland marsh (A). As indicated, the upland marsh appears to out preform a low-land marsh when related to resurgence of wetland species; nevertheless, this variation is not statistically valid.

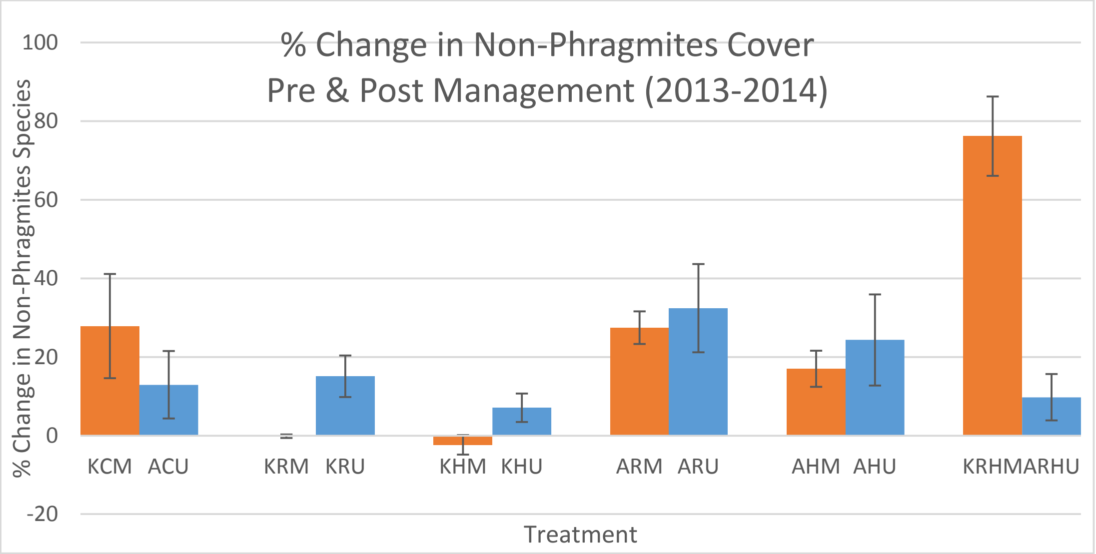


Figure 14: This bar graph signifies the average change (%) in non *Phragmites* coverage per plot. Positive values denote the average resurgence (%) of non-*Phragmites* wetland species.

Common Name	Scientific Name
Arrow Arum	*Peltandra virginica* (L.) Schott
Blackwillow	*Salix nigra* Marsh.
Broadleaf Cattail	*Typha latifolia* L.
Buttonbush	*Cephalanthus occidentalis* L.
Climbing hempvine	*Mikania scandens* (L.) Willd
Duck Potato	*Sagittaria latifolia Willd*
Groundnut	*Apios americana* Medik.
Japanese Honeysuckle	*Lonicera japonica*
Jewelweed	*Impatiens capensis* Meerb.
White Mulberry	*Morus Alba*
Narrowleaf Cattail	*Typha angustifolia* L.
Pickerelweed	*Pontederia cordata* L.
Porcelainberry	*Ampelopsis brevipedunculata (Maxim.) Trautv.*
Primrose Willow	*Ludwigia peplodes*
Purple Loosestrife	*Lythrum salicaria* L.
Rice Cutgrass	*Leersia oryzoides* (L.) Sw
Rose Mallow	*Hibiscus moscheutos* L
Smartweed	*Polygonum sp.*
Spatterdock	*Nuphar lutea*
Tickseed	*Bidens sp.*
Water Hemp	*Amaranthus cannabinus* (L.) Sauer
Wild Grape	*Vitis sp.*

Table 7: Represents the plants species that reemerged as a result of *Phragmites* mortality.

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