# HYDROLOGY, RESTORATION TECHNIQUES AND EMERGENT VEGETATIVE MATS: EFFECTS ON NATIVE VEGETATION AND REED CANARY GRASS AT THREE WESTERN WASHINGTON OREGON SPOTTED FROG SITES

by

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A Thesis Submitted in partial fulfillment Of the requirements for the degree Master of Environmental Studies The Evergreen State College June 2021 ©2021 by Naomi Korchonnoff. All rights reserved.

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<u>11 June 2021</u>

#### ABSTRACT

#### HYDROLOGY, RESTORATION TECHNIQUES AND EMERGENT VEGETATIVE MATS: EFFECTS ON NATIVE VEGETATION AND REED CANARY GRASS AT THREE WESTERN WASHINGTON OREGON SPOTTED FROG SITES

#### Naomi Korchonnoff

Anthropogenic forcing, climate change, and the spread of invasive species have extirpated the Oregon spotted frog (Rana pretiosa: OSF) from much of its historical range. The invasive species Reed canary grass (Phalaris arundinacea: PHAR) outcompetes low stature vegetation in the shallows of open-canopy, low-elevation emergent wetlands where the OSF breeds, posing formidable challenges to its conservation. The biodegradable coconut-coir emergent vegetative mat (EVM) is an integrative restoration method not yet evaluated for ecological use in the Pacific Northwest. From 2017 to 2019, this study evaluated the effects of pre-vegetated EVMs installed at different water depths using three different restoration techniques (solarization, herbicide, mowing) on native vegetation and PHAR cover (%) at three OSF restoration sites near Olympia, Washington. To better understand the effectiveness of EVMs as part of an integrated wetland restoration strategy for OSF habitat, I sought to answer four questions: 1) How effective are EVMs at establishing native vegetation? 2) How effective are EVMs at suppressing PHAR cover? 3) Which restoration treatment methods were most effective at removing PHAR prior to EVM placement? and 4) Which hydrological zones were associated with the best outcomes? Statistical analyses using a Bayesian approach to the Hierarchical Beta-Regression with a logit transformation revealed minor differences between the restoration treatments and hydrology depths on native vegetation and PHAR cover over time in the EVMs, with one pre-planted EVM emergent species (Spreading rush [Juncus supiniformis]) showing a positive response to the EVM treatment in deep and shallow hydrology. The results of the herbicide (1% imazapyr) and mowing treatment supported preexisting evidence of its effectiveness at controlling perennial weeds. In sum, EVM effectiveness was limited under the application regimes used, and the cost tradeoff to apply it relative to alternative methods was poor. The EVM may not be suitable for OSF habitat restoration where PHAR is well-established, but future studies could evaluate its effectiveness when paired with targeted, possibly herbicidal, and more strategic retreatments.

Keywords: wetland restoration; wetland ecology; invasive species; amphibians; Reed canary grass; Oregon spotted frog; mowing; herbicide; solarization; emergent vegetative mat; wetland plants; Pacific Northwest

### **TABLE OF CONTENTS**

TABLE OF CONTENTS	iv
LIST OF FIGURES	vi
LIST OF TABLES	vii
Acknowledgements	viii
CHAPTER 1: LITERATURE REVIEW	
A Brief History of Wetlands Restoration in the United States	
The Oregon Spotted Frog: Status and Ecology	
Reed Canary Grass	9
Range, Genetics, Morphology & Phenology	9
Environmental Effects	
Wetland Restoration Methods in Context of Reed Canary Grass Abater Spotted Frog Habitat Conservation	nent for Oregon 
Mechanical Methods	
Herbicide	
Prescribed Burning	
Grazing	
Solarization	
Biological Control	
Hydrological Restoration	
Revegetation & Native Species Selection	
Other Shade Treatments	
Amphibian Responses to Restoration Methods	
Alternative Methods	
Conclusion	
CHAPTER 2: RESEARCH MANUSCRIPT	
Materials and Methods	
Study Sites	
EVM Production	
EVM Placement	

Restoration Treatments	
Mima Creek	
Watkins Marsh	
West Rocky Prairie	61
Monitoring	61
Data Analysis	
Results	
Native Species Response by Site, Depth, and Year	
Reed canary grass (PHAR) Response	
Non-target Native and Exotic Species Richness	71
CHAPTER 3: DISCUSSION & CONCLUSION	
Discussion	
Conclusion	
Reference List	
Appendices	
Appendix A	
Appendix B	
Appendix C	

### LIST OF FIGURES

Figure 1 OSF Historical Range and USFWS Designated Critical Habitat	
Figure 2 EVM Study Site Locations	
Figure 3 EVM Placement by Site	59
Figure 4 Emergent Vegetation Mat (EVM) Schematic Sampling Design	63
Figure 5 2017 Hydrology Depths and Temperature by Site	64
Figure 6 EVM Treatment Effects on Native Species Cover (%)	
Figure 7 EVM Treatment Effects on PHAR Cover %	69
Figure 8 Beta Regression Results for EVM Treatment Effects on PHAR	

### LIST OF TABLES

Table 1 Monitoring Protocol for 2017–2018 and 2019	62
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#### Acknowledgements

First, I'd like to thank the organizations that were involved in this research project: Washington Department of Fish and Wildlife, the U.S. Fish and Wildlife Service, Center for Natural Land Management, and Sustainability in Prisons Project through the Evergreen State College. I thank my thesis reader, Sarah Hamman, for her many edits and revisions to my drafts over the past two years, which I am fairly certain exceeded what is typically expected of her role as a faculty reader. I owe much gratitude to Marc P. Hayes (WDFW), who contributed significantly to this project from start to finish and was integral to my success on several accounts. M. P. Hayes facilitated the processing and final analysis of a dataset that turned out to be highly complex, and provided substantive review of my work, mentorship, and technical training to me since 2019.

I'd also like to thank others who assisted with project funding, monitoring, editing, and *en lieu* of M. P. Hayes' mentorship, including: Ilai Karen (WDFW) for assisting with the final analysis, Carl Elliott (SPP) for allocating 2019 monitoring funding, Amber Martens (FWS-JBLM) for her direction with 2019 monitoring, volunteer (intern) assistance, and Watkins Marsh management, study design, and communication. In particular, I want to highlight how important John Withey's assistance with communicating the analysis was in this project. Special thanks to the Evergreen State College Master of Environmental Studies (MES) program faculty, student assistants and members of my cohort, and of course all the JBLM volunteers and staff (Saff Killingsworth) and other CNLM staff who helped with my data collection in 2019. Thanks to S. Hamman for allocating these resources to assist with the completion of this project.

Additionally from the MES faculty, I thank John Kirkpatrick (Young John) for his feedback on initial drafts of my project, as well as his encouragement and advice on all things R related, Shawn Hazboun for emboldening me to become a better writer, Mike Ruth for refining my inspiration and passion for GIS through solid instruction, enthusiasm, and his dedication to individual student success, and Kevin Francis (MES Director on Sabbatical Leave 2020-2021), who displays an ethical clarity in his leadership role that, while often overlooked, is tantamount to a future that will be truly equitable. K. Francis initially introduced me to my thesis project and helped connect me with some of the most influential people in my career.

I cannot thank my friends (notably: Kristin, Allison and Anna) enough for being the light at the end of the tunnel for me during my final year, as well as my loving mother Sharon, brother Mike, and my adoring, half-retired work dog Remi for their support and friendship during this process.

And last but certainly not least, I'd like to extend my gratitude to J.Withey and newly appointed Assistant Director, Averi Azar for their leadership, hard work, and empathic dedication to the success of the MES class of Fall 2018 – Spring 2020/2021through a very trying time in modern history.

#### **CHAPTER 1: LITERATURE REVIEW**

This literature review summarizes wetland restoration research from the mid-1990s to present with a focus on trends in invasive species control methods used for *Phalaris arundinacea* (Reed canary grass: PHAR) management in palustrine-emergent wetlands. A brief overview of the history associated with palustrine-emergent wetlands loss, mitigation and restoration will be provided as context for the importance of invasive species control in the United States (U.S.). As a pretext for the evaluation of an alternate restoration method, the invasive mechanisms of PHAR are examined in context of its abatement in critical habitat restoration areas for the focal species of this thesis, *Rana pretiosa* (Oregon spotted frog: OSF).

These topics are followed by an overview of trends in restoration science highlighting studies that have employed the most used restoration methods for PHAR control, including herbicide, mowing, tilling, solarization, or some combination of at least two of these. Finally, as a precursor for this thesis on the effects of different restoration treatments, hydrological gradients, and native species plantings on PHAR cover with the emergent vegetative mat (EVM), I provide an overview of existing literature on integrated pest management strategies.

#### A Brief History of Wetlands Restoration in the United States

A historical synopsis of the political landscape leading to wetlands losses and subsequent restoration efforts in the U.S. shows how advances in restoration science have shaped the contemporary landscape. Wetland alteration, conversion, and destruction has occurred more rapidly in the U.S. than any other developed nation in the world based on total wetland acreage lost, numbers of wetlands, and wetland density since before European colonization of North America (Prudham, 1998; Quesnelle et al., 2013). Since the early 1700s, wetland area in the U.S.

has been reduced to remnant fragments of extant watersheds that once encompassed large, interconnected systems throughout the contiguous U.S. These wetlands hosted diverse vegetation assemblages that supported the original biodiversity of the nation (Kneller et al., 2018; Quesnelle et al., 2013; Thorslund et al., 2017).

In many ways, the present status of compositional changes to the quality and quantity of wetlands today are legacies of post-Vietnam era policies that shaped the trajectory of permissory wetlands development after the drafting of the Classification of Wetlands and Deepwater Habitats of the U.S. in 1979 (Cowardin et al., 1979). Throughout the greater portion of the 20<sup>th</sup> century, wetlands had remained largely unprotected, and no comprehensive national plan existed for the conservation of natural resources and wildlife until the 1970s with creation of the Environmental Protection Agency (EPA) in 1970, passage of the Clean Water Act (CWA) of 1972, and the initial release of the Endangered Species Act (ESA) under President Richard Nixon in 1973 (Corn & Wyatt, 2016; Cowardin et al., 1979; Dwire et al., 2018).

In 1987, the EPA intervened in wetlands regulation in the National Wetlands Policy Forum (NWPF), altering wetland regulation language to prioritize acreage and area in a "no net loss" policy (Bendor, 2009). Some posit that the wetland rating valuation system permits indiscriminate habitat destruction by scaling wetland ratings under economic metrics of size and scale rather than evaluating them under the best available science (Bendor & Riggsbee, 2011). Proponents argue that by categorizing wetlands by their perceived value under the national wetland rating system, greater permissory wetlands loss has occurred than before the amendment to Section 404 of the CWA (Quesnelle et al., 1999).

The widespread loss of temporal wetland types is believed to have resulted in part from their denigrated functional and compensatory status under the U.S. EPA wetland rating system,

wetland classification system, and functional structure of wetlands mitigation policy (Adamsuilli, 2015; Bendor, 2009; Whigham, 1999). Many have argued that compliance with the wetland regulatory environment enters bias into permissory wetland development under the CWA Section 404 permitting process (Bendor & Riggsbee, 2011; Holland et al., 1995). Increased attention to the broad scale effects of these landscape changes on palustrine-emergent wetlands suitable for amphibian species should be incorporated into research, management, and restoration objectives.

#### **The Oregon Spotted Frog: Status and Ecology**

The OSF is an at-risk anuran species endemic to the Pacific Northwest that has distinct hydrological and structural (mostly vegetative) requirements. As an ectothermic 'true frog' of the family *Ranidae*, it is endemic to the Pacific Northwest of the North America, historically ranging from northern California to Southern Vancouver, British Columbia (B.C.) (Hallock, 2013) (**Error! Reference source not found.**).

In 1997, the OSF was listed as Endangered in Washington State (McAllister & Leonard, 1997). Subsequently, B.C. listed the species as Endangered in 2000 (COSEWIC, 2011). In 2014, the U.S. Fish & Wildlife Service (USFWS) listed the OSF as Threatened under the Endangered Species Act (USFWS, 2014).

Anthropogenic forcing, climate change, and the spread of invasive species have extirpated the OSF from much of its historical range. The decline of the OSF is primarily attributed to habitat loss, predation both by native and invasive aquatic vertebrates, and exotic plant invasions (Hayes, 1997; Hayes et al., 2012). As a fully aquatic species, the OSF stands out among the true frogs of the Pacific Northwest in that it breeds in shallow water, it spends its



Figure 1 OSF Historical Range and USFWS Designated Critical Habitat

*Note.* Pacific Northwest range of the OSF: Historical range of the OSF (orange polygons) from Northern California to Southern Vancouver, B.C. U.S. Fish & Wildlife Service designated critical habitat areas (blue) indicate proposed critical OSF conservation units (M. P. Hayes, personal communication, 5 June 2021; USFWS, 2014).

non-breeding active season in low emergent marsh and it overwinters in freeze-protected, oxygen-rich waters (Hayes, 1997; Loman, 2002; McAllister & White, 2001; Pearl & Hayes, 2004). These habitat requirements make necessary a juxtaposition of these aquatic habitat variables in order to complete its seasonal life cycle (Hallock, 2013; Hayes et al., 2003; Loman, 2002; McKibbin et al., 2008; Pearl et al., 2009; Watson et al., 2000). For example, ephemeral wetlands that support OSF oviposition often occur in proximity to hydrologically permanent and deeper waters that support adults and juveniles during the non-breeding active season (Hayes, 1997; Loman, 2002; McKibbin et al., 2008; Watson et al., 2000). Further, strong evidence exists that hydrological features likely need to be interconnected within 1100 m for seasonal movements and migration of post-metamorphic life stages, especially during periods of drying (Hayes, 1997; Pearl et al., 2018).

The OSF is especially sensitive to thermal changes resulting from tall, shade-bearing vegetation. For example, shading of oviposition habitat has been shown to decrease the water temperature of oviposition shallows (Hayes, 1997; Kapust et al., 2012; Pearl et al., 2005; Pearl & Hayes, 2004, 2005). Thus, low-stature emergent vegetation is essential for OSF oviposition and larval development in early stages, providing oviposition pools and access to sunlight required to thermally regulate egg-masses to temperatures above twenty degrees Celsius ( $\geq$  20 °C) (Hayes, 1997; McAllister & White, 2001; Pearl & Hayes, 2004). Emergent vegetation may also screen

OSF egg-masses and metamorphosed tadpoles from predators (Henning & Schirato, 2006). These habitat features appear important since their absence can result in significant mortality, which may place extant populations of this struggling amphibian at risk (Hallock, 2013; Hayes et al., 2006, 2012; McAllister & Leonard, 1997; Pearl & Hayes, 2004; Pearl et al., 2009).

Unfortunately, few connected wetland systems that support OSF habitat characteristics remain. Many have lost key characteristics such as landscape variation, habitat connectivity, and hydrological gradients that are essential for anuran survival (Henning & Schirato, 2006; Loman, 2002; Stuart et al., 2003). In Washington State, critical habitat for amphibians in the south Puget Sound region featuring open-canopy, graminoid and emergent dominant species assemblages have been steadily declining for the past half-century or more (Miller-Adamany et al., 2019; Robertson et al., 2018). Land fragmentation from deforestation in the Pacific Northwest has accounted for the greatest proportion of conterminous U.S. land cover change patterns compared to anywhere else in the States by a factor of 2:1 (50% of all land use pattern changes) (Homer et al., 2020).

Habitat connectivity, hydrology, and plant community structure are important to OSF reproduction, overwintering habits, community dynamics, and genetic diversity (Brown et al., 2012; Hayes, 1997; Hayes et al., 2001; Loman, 2002; Watson et al., 2000). Further, reliably permanent hydroperiods have been identified as crucial to post-metamorphic juvenile survival (Hallock, 2013). Both water temperature and depth during and after oviposition are critical for larval development and survival (Hayes, 1997; Hallock, 2013; Hayes et al., 2001; Watson et al., 2000). Cold snaps and freezes late during oviposition can kill larva (Pearl & Hayes, 2004; Pearl et al., 2009). The lethal minimum threshold temperature for juvenile and larval specimens is  $\leq 6$ °C (Bowerman & Pearl, 2010; Rowe et al., 2021), but compelling evidence exists for the survival of adult specimens during winter dormancy at or below the presumed lethal minimum temperature (Bowerman & Pearl, 2010).

Landscape fragmentation disproportionately affects the OSF compared to other true frog species, in part by degrading many of the habitat features that support their multiple life stages (Hayes, 1997; Kapust et al., 2012; Robertson et al., 2018). The OSF is known to return to remnant breeding sites for generations, and therefore is likely less adaptable to habitat alteration or destruction than other species of true frogs in the Pacific Northwest (Hayes, 1997; Kapust et al., 2012; Loman, 2002). One metric that illustrates this can be observed in movement distances of newly metamorphosed and adult frogs from the site of oviposition to feeding and overwintering habitat. The OSF travels across less area on average than other Pacific Northwest ranid species, with seasonal migration lengths measuring between two hundred to five hundred meters (200m – 500m) on average, with greater distances of at least 1145 m (1.15 km) recorded in some cases (Hayes, 1997; Pearl et al., 2018; Robertson et al., 2018). In tandem with the dramatic reduction of its habitat, remnant OSF populations are frequently isolated within fragments of their historic range, leading to potential genetic complications from interbreeding (Hayes, 1997; Hayes et al., 2006; Pearl et al., 2009).

Other factors underlying amphibian declines have affected the OSF, including negative responses primarily observed in juveniles to chemical pollution, nutrient loading, and water chemistry changes from agricultural runoff and other non-point sources, predation by invasive species, diseases and pathogens, and the effects of climate change on weather patterns, hydroperiods, and water temperatures (Blaustein et al., 2001, 2020; McKibbin et al., 2008; Reeves et al., 2016). Some of these have been shown to be of less concern. For example, the etiological agent of chytrid fungus *Batrachochytrium dendrobatidis* has been demonstrated to

pose little risk to juvenile OSF (Padgett-Flohr & Hayes, 2011), likely a function of unique skin peptides in OSF (Conlon et al., 2011).

#### **Reed Canary Grass**

Reed canary grass is a fast-growing invasive grass of the family *Poacae* that exhibits superior phenotypic plasticity, adaptative responses to climate extremes, and high fecundity in comparison to most of its native and invasive North American competitors (Anderson, 2019; Hayes et al., 2013; Kercher et al., 2006; Nelson et al., 2014). Its most prevalent variant (*Phalaris arundinacea*), derived from the North American legacy genotype *P. arundinacea* var. *picta* (i.e., Ribbon grass), readily clones from rhizomes during vegetative propagation. Once established, this species is exceedingly difficult to control as it is prone to reinvade newly restored areas in the advent of flooding or other disturbances, including those resulting from invasive species control methods themselves (Iannone & Galatowitsch, 2008a, b). A basic problem is that over the past century PHAR has had contradictory uses, where on the one hand, it has been the focus of control as an invasive, while simultaneously being grown as forage for livestock, promoted as a species to limit bank erosion, and marketed as an ornamental for household use (Clark & Thomsen, 2020; Kávová et al., 2018). These contradictory uses present a daunting challenge to its effective management.

#### Range, Genetics, Morphology & Phenology

Even though PHAR is assumed to be a European, Asian, and North American-endemic species, its global range has expanded considerably since the beginning of the 20<sup>th</sup> century with the development of its cultivation for forage crop and erosion control purposes (Casler, 2010; Klaas et al., 2019). Over the past century, PHAR has become circumboreal in distribution— ubiquitous in all continents except for Greenland and Antarctica, occurring in North and South America, East and South Asia, Europe, the Middle East, Africa, and Australia (Casler, 2010; Lavergne & Molofsky, 2006). Despite its worldwide prevalence, PHAR has yet to expand into

the growing terrestrial extremities of the north or south poles, though this is subject to change as terrain is increasingly exposed from anthropogenic-caused climate warming (Green & Galatowitsch, 2001).

For reasons not well understood, North American cultivars of PHAR have become more invasive and attained a greater range in the past twenty to thirty years (Casler, 2010; Halliday, 2013; Klaas et al., 2019; Molofsky et al., 2017). Perspectives on PHARs invasiveness vary by country and sometimes within regional subdivisions, but one noticeable trend in the literature (chiefly from sites far north or south of the equatorial line) is the documentation of anthropogenic impacts on its spread. For example, in Quebec, B.C., large expanses of wetland areas dominated with PHAR were observed between 1963 to 1978, but little data exists on its distribution in the provinces before the 20<sup>th</sup> century, so interpreting historical changes will require special techniques. The modern expansion of PHAR in Quebec was ultimately attributed to human cultivation of the species, nitrate pollution, altered water level fluctuations, and road construction in Canada (Anderson, 2019; Lavoie et al., 2005).

Conversely, the catalyst for PHAR's invasive spread in North America has been widely contested in U.S. restoration ecology literature (Adams & Galatowitsch, 2006; Kávová et al., 2018; Pavlegio & Kilbride, 2000). North American literature has tended to link PHAR's superior phenotypic expression to inadvertent breeding between U.S. endemic types that were cultivated for forage crop and erosion control since the early 20<sup>th</sup> century, and 19<sup>th</sup> century introduced Eurasian types (Adams & Galatowitsch, 2006). It was thought that the rapid invasion by North American PHAR subvarieties were a product of its inadvertent hybridization with presumably more invasive European types (Galatowitsch et al., 1999; Lavergne & Molofsky, 2007; Pavlegio & Kilbride, 2000).

Although native PHAR genotypes are confirmed to have been present in the U.S. and Canada before the introduction of European variants, multiple publications in the early 2000s continued to support this theory, citing a cluster of its early proponents throughout restoration ecology literature (Adams & Galatowitsch, 2006; Lavergne & Molofsky, 2007; Nelson et al., 2014; Perry & Galatowitsch, 2003; Rojas & Zedler, 2015). This assumption was not only limited to theory, despite the lack of a reference genome or complete transcriptome sequencing to comprehensively assess its speciation before the 2010s (Haiminen et al., 2014). Consequently, field and laboratory studies have typically introduced the Euro-invasion theory in a variety of publications with differing objectives, though few give equitable attention to its domestic proliferation in the U.S. for erosion control, cover crop stock, and ornamental purposes that preceded research on its invasiveness (Adams & Galatowitsch, 2006; Lavergne & Molofsky, 2004; Lindig-Cisneros & Zedler, 2002).

Others have attributed the occurrence of PHAR's rapid 20<sup>th</sup> century expansion to some of the broadscale landscape changes incurred by colonialists in post WWII America (Jakubowski et al., 2010, 2011; Price et al., 2018). These impacts largely stemmed from the spread of agricultural practices and land use changes co-occurring with the genocide and displacement of aboriginal Americans, the massive extent of wetlands conversion, alteration from nutrient loading by irrigation and fertilization practices, and the reduction of remnant hydrophytic wetland species assemblages that once occurred in great abundance across the vast lowland swaths of the inter-continental U.S.—before the major impacts of river damming, groundwater depletion, and other hydrological manipulations homogenized the natural features of the Great Plains (Jakubowski et al., 2010, 2011). Proponents of this perspective stipulate that widespread wetland alteration and conversion have been the leading factor in PHAR's invasiveness in North

America, while still others adopt the alien-invader hypothesis as PHAR's initial catalyst as precedent to its modern expansion (Rojas & Zedler, 2015). The Great Depression era proliferation of PHAR for its extreme drought resistance has also been implicated in the spread of this species (Anderson, 2019; Casler, 2010). Some of the most antiquated reports confirm European germplasms were introduced to the U.S. as early as 1835 for hay and pasture forage grass (Jakubowski et al. 2013). In the Pacific Northwest, PHAR introduction and subsequent spread was alleged to have occurred in the early 1900s following its intentional propagation as a cover crop by foresters seeking to enrich the soils of recently harvested timber units, though numerous contradictions exist in the literature regarding whether it is really endemic to the Northwest (Adams & Galatowitsch, 2006; Kercher et al., 2006). Furthermore, acknowledgement of the cultural significance of PHAR for many Native American tribes has been recognized by some scholars as a knowledge gap in PHAR's pre-European endemicity and early spread that warrants further investigation.

Some North American PHAR variants are now known to be genetically distinct from European strains, having differentiated in sympatry over time while in proximity to each other (Anderson, 2019; Jakubowski et al., 2013; Kávová et al., 2018; Nelson et al., 2014). Following concurrent advances in genomics sequencing for bioproduction of renewable energy sources, PHAR has attracted global attention for its promising phytoremediation and bioengineering potential. Phenotypic plasticity and high genetic diversity among PHAR's North American variants have increased cultivation of the species in the green technology industry since the release of CRISPR genomic editing software (Haiminen et al., 2014; Iannone & Galatowitsch, 2008; Kávová et al., 2018; Nelson et al., 2014). These findings can be partially credited to the seminal discovery of the Cas9 gene ('spacer') in 2012, and the subsequent invention of a

software program named after clustered regularly interspaced short palindromic repeats (CRISPR<sup>®</sup>) that spurred a revolution in modern genomics sequencing (Jinek et al., 2012; Kávová et al., 2018). Consequently, PHAR is one of the most well studied plants in the world for its potential in genetics research, cultivation as a biofuel product, and wastewater treatment stock species (Casler, 2010; Iannone & Galatowitsch, 2008a; Klaas et al., 2019).

Some misgivings exist about genetic engineering practices in the U.S. due to the perception that such activities may increase the range of cultivated invasive species. It should be noted, however, that at least one transcriptome sequencing genetic study shed doubt on the possibility of PHARs invasiveness because of intentional biological modification (genetic engineering) (Jakubowski et al., 2011). Though varied perspectives on PHARs invasiveness in North America exist, some studies on PHAR genomics have pointed to the likelihood of human-aided mechanisms in its spread, noting the frequent occurrence of high-quality wetland losses and destructive agricultural practices that have led to widespread nutrient loading and erosion in wetlands (Casler, 2010; Molofsky et al., 2014).

#### Environmental Effects

PHAR thrives particularly well in the temperate climate of the Pacific Northwest due to the characteristically high annual rainfall and long growing season (Galatowitsch et al., 1999). Infestations of the invasive grass dominate many seasonally inundated wetlands in the Pacific Northwest (Perry et al., 2004a, b; Pearl & Hayes, 2004). North American genotypes are clonal, often forming dense monocultures that can easily compete with most native wetland species for dominance, as well as many invasive species (Kercher et al., 2006). The distinctive height, density, and litter accumulation characteristic of PHAR make it stand out in its non-native colonial stands (Eppinga et al., 2011). It typically grows three to six feet tall from upright,

hairless, hollow culms, beginning its new growth in the early spring and persisting through early to mid-fall (Reinhardt Adams & Galatowitsch, 2005). Reproduction is both sexual and asexual, occurring by seed dispersal that is wind or water-borne, and clonally by rhizome (Casler & Undersander, 2006). North American phenotypes produce prolific root to shoot biomass, with rhizomes reaching depths of up to one to two feet in well-established monocultures. Seed fecundity is reduced in large infestations due to mat-forming litter the plant accumulates over time, often referred to as 'thatch' (Eppinga et al., 2011; Hayes et al., 2013; Kaproth et al., 2013).

Even though PHAR produces as many as six hundred seeds per panicle, its spread by seed is less important to its homogenization than that of its rhizomatous vegetative propagation (Casler & Undersander, 2006). Regardless, PHAR seeds can remain viable for over twenty ( $\geq$  20) years in the soil, which significantly increases its prevalence in the seed bank in relation to other species over time (Clark & Thomsen, 2020). Seeds can germinate under low light conditions, but this advantage declines sharply once shading from canopy cover falls below fifteen percent (15%) of a given area (Lindig-Cisneros & Zedler, 2002).

Restoration efforts are significantly hampered by PHAR's ability to outcompete most native plants by allocating carbohydrates to its culms, supporting stem density, vegetative height, and rhizomatous spread (Martinez & McDowell, 2016). Multiple studies have affirmed that PHAR responds to shade competition from taller species by allocating carbohydrates to adventitious roots under very low-light conditions, resulting in the continued survival and spread of the species following shifts in canopy cover (Iannone & Galatowitsch, 2008b; Maurer et al., 2003). The rapid clonal spread of PHAR allows it to monopolize space earlier than its competitors, attaining culm (basal stem) biomass of up to one-hundred and sixty-five grams per plant (165 g/plant) and four-hundred and twenty-six grams per plant (426 g/plant) within two

years, respectively (Hayes et al., 2013; Reinhardt Adams & Galatowitsch, 2005). The advantage PHAR gains through spatial pre-emption enables it to establish long before other competitors germinate or attain greater height by allocating sugars to post-dormant tillers before the last frost of the growing season occurs (Hayes et al., 2013).

As a cold-season perennial, PHAR is efficient at adapting to some of the effects of climate change that are being experienced in North America (Haiminen et al., 2014; Kávová et al., 2018; Lavergne et al., 2010). Moreover, PHAR has a C3 photosynthetic pathway that induces thermal regulation at both low elevations and high altitudes, cold and hot temperatures, and when exposed to prolonged drought stress or flooding (Waring & Holaday, 2017; Zheng et al., 2017). The photo-inhibition mechanisms PHAR has adapted to resist the effects of high photosynthetic active radiation (PSII) via thermal respiration are expressed in its highly temperature reflexive stomata and carbohydrate translocation in response to radiative forcing that has even been observed at high altitudes (Zheng et al., 2017). Further, PHAR's C3 photosynthesis-pathway adds to its competitive advantage over most plants in North America, where climate change is manifesting in hotter, longer summers and milder winters (Waring & Holaday, 2017). Warmer, wetter conditions and recurring mild winters have led to higher PHAR biomass yields and are expected to support its continued expansion under projected climate change (Kávová et al., 2018; Reinhardt Adams & Galatowitsch, 2005; Waring & Holaday, 2017).

The genetic plasticity of North American PHAR phenotypes aid in its rapid colonization of wetlands that are connected by hydrology, experience intermittent or annual natural flooding, or are otherwise exposed to disturbances from human activities such as agricultural nutrient loading and stormwater pollution (Gerard et al., 2008; Kapust et al., 2012; Rojas & Zedler, 2015; Sonnier et al., 2018; Zedler & Kercher, 2004). In the Pacific Northwest, PHAR has adapted to a

wider range of hydrological conditions than any of its competitors excepting *Typha latifolia* (Broadleaf cattail). This morphological advantage is apparently enhanced by its anoxia-tolerant rhizomes, hollow culms retaining thin pith for flexibility and growth under varying inundation levels (Hayes et al., 2013; Kercher & Zedler, 2004). It can survive in deep water between twenty-five to thirty-five centimeters, within a margin of eight ( $25 \pm 8$ cm, max 35cm), and at depths of up to eighty centimeters ( $\leq 80$  cm) by elongating vertical culms to outpace competitors and developing dense adventitious rhizomes that increase in response to shade and soil disturbance (Coops et al., 1996; Zedler & Kercher, 2004). Further, in water over thirty centimeters ( $\geq 30$  cm) deep, hydrochory in PHAR has been documented (Coops et al., 1996). In the advent of hydrochory, the detached PHAR fragments form independent clonal mats that can travel across river channels, lakes, marine confluences, and other contiguous bodies of water, starting new infestations in terrestrially estranged regions (Zedler & Kercher, 2004).

In lowland emergent-palustrine wetlands, floodplains, and estuarine habitats colonized by monospecific stands of PHAR, the geomorphological characteristics of riparian systems (i.e., water margins, lacustrine and riverine banks, river sinuosity) can change dramatically from the erosion PHAR causes (Martinez & McDowell, 2016). Over time, PHAR monopolizes below-ground space as it invades, dissolving the root structure of native vegetation that holds banks in place along rivers, streams, lakes, and other terrestrial-freshwater margins (Catford et al., 2011; Martinez & McDowell, 2016). Thus, PHAR's biotic homogenization (BH) can potentially alter large-scale ecological processes by depleting humic content and arbuscular mycorrhizal fungi (AMF), as well as native root systems, eventually causing banks to collapse from the loss of soil integrity (Kneller et al., 2018; Molofsky et al., 2014; Weilhoefer et al., 2017).

Finally, a significant portion of the literature explores the biological conditions behind PHAR invasiveness, including the role of nutrient addition in either reducing or encouraging PHAR cover (Eppinga et al., 2011; Jakublowski et al., 2011; Klaas et al., 2019; Lavergne et al., 2010; Magee & Kentula, 2005; Maslova et al., 2007; Miller-Adamany et al., 2019; Molofsky et al., 2014, 2017; Price et al., 2018; Schooler et al., 2009; Waring & Holaday, 2017). Several studies have examined the effects of nitrogen (N) availability, as it induces a strong positive response in PHAR's invasive proclivity (Green & Galatowitsch, 2001; Iannone et al., 2008a, b; Perry et al. 2004a). Many wetland habitats exposed to unregulated N deposition from agricultural activities in the U.S. since the 1980 are also invaded by PHAR (Galatowitsch et al., 1999; Kercher et al., 2004).

PHAR monocultures can alter vegetation composition and soil health, which in turn affects the herbivory rates and diversity of arthropod communities (Weilhoefer et al., 2017). Changes in soil microbiota that affect the diversity and quantity of detritivore assemblages (i.e., nematodes and fungi) can result from the alteration of soil composition and the proportion of essential nutrients (i.e., N, K, P), ultimately impacting certain species of native plants that require symbiotic relationships to survive (Porazinska et al., 2014; Pritekel et al., 2008). This is partly accomplished through decomposition of PHAR's annual thatch layer, providing high soil-N content that boosts tiller growth the following season (Iannone et al., 2008b). Further, PHAR homogenizes space and eliminates competitors by limiting light availability and monopolizing N uptake (Eppinga et al., 2011). These alterations in vegetation composition and soil health create positive feedbacks for PHAR invasions with monospecific soil biota, making it exceedingly difficult for symbiotic-dependent species to compete for cover. The introduction of human added admixtures such as C amendments (sucrose, sawdust) have been proposed as solutions to PHAR's early adventitious growth when applied with correct timing and material type, but this is a method that could benefit from further investigation (Iannone et al., 2009; Porazinska et al., 2014).

## Wetland Restoration Methods in Context of Reed Canary Grass Abatement for Oregon Spotted Frog Habitat Conservation

Wetland restoration studies have historically prioritized the preservation and restoration of upland, terrestrial riparian habitats rather than lowland palustrine-emergent wetlands (Adusumilli, 2015; Bendor, 2009). While some research has been conducted on the effects of integrated vegetation management in the context of invasive species abatement for amphibian conservation, few studies have evaluated the issue of hydrology in combination with multiple habitat restoration methods for OSF habitat needs specifically. Additionally, fewer studies have examined low-elevation palustrine-emergent wetlands restoration than upland forested riparian and scrub-shrub wetlands restoration (Kettenring & Adams, 2011; Zedler & Rojas, 2014). In the 1990s, shallow, ephemeral, palustrine-emergent marshes were more frequently developed than permanently inundated, upland-forested, and marine or estuarial wetlands (Holland et al., 1995). At present, lowland palustrine-emergent wetlands, inland wet prairie bogs, and rich fens are proportionally more degraded or destroyed than other wetland types in the U.S. (Young et al., 2005).

From the early 2000s to present, a call for open-source data and information sharing among land managers, researchers, and restoration practitioners consistently surfaced in the literature (Green & Galatowitsch, 2001; Horvath et al., 2017; Kettenring & Adams, 2011; Lavergne & Molofsky, 2006; Lengyel et al., 2020; Raimundo et al., 2018; Taddeo & Dronova, 2020; Thorslund et al., 2017; Young et al., 2005). Often, management strategies are isolated to

specific restoration objectives that are contingent upon grant funding limitations according to species group, habitat type and federal protection ranking, or under specific timelines and budget constraints. Consequently, negative outcomes may occur with quick decision making that is largely based upon limited knowledge from a few disparate studies, in part due to the lack of comprehensive repositories of shared knowledge about biogeographically relevant restoration data (Adams & Galatowitsch, 2006; Raimundo et al., 2018; Thorslund et al., 2017). Some scholars argue that these policies have ultimately contributed to a pattern of repetitive, costly restoration outcomes and spurious decisions from within a regulatory landscape that is often governed by constraints imposed upon land managers from time and funding limitations (Adamsuilli, 2015; Hierro et al., 2005).

Many studies exist on the utilization of traditional invasive species management strategies for PHAR control, both from a wetland restoration perspective as well as within the broader context of the field itself. The continued demand for research on PHAR control is dually influenced by the urgent need to address research gaps in older methods that have broadly failed to stave off its global spread, and the less explored trends in restoration research towards more adaptive, sustainable, and ecoregion focused treatment approaches that have yet to be fully evaluated (Kercher et al., 2007; Raimundo et al., 2018). Solarization in combination with other control methods has experimental claims to be one of the most effective treatment methods for reducing PHAR's rhizome biomass (Gerard et al., 2008; Pfeifer-Meister et al., 2012b; Tu & Salzer, 2005) outside of the more comprehensive literature on systemic herbicide use for PHAR control (Adams & Galatowitsch, 2006; Bahm et al., 2014; Foster & Wetzel, 2005; Paveglio & Kilbride, 2000; Pfeifer-Meister et al., 2012a, b; Yahnke et al., 2013).

Over the past three decades, PHAR has emerged as an apex invader in North America, Europe, and East Asia, for which control methods have been extensively documented both in published field experiments and laboratory mesocosms (Healy & Zedler, 2010; Jakubowski et al., 2010; Kettenring & Adams, 2011). Intensive focus exists in the literature on the implications of PHAR's genetic plasticity and its hybridization in North America and Europe, which contributes to an understanding of the invasive mechanisms that underpin infestations on a regional basis (Coops et al., 1996; Eppinga et al., 2011; Galatowitsch et al., 1999; Jakubowski et al., 2010; Matthews et al., 2020; Price et al., 2018; Richardson et al., 2007; Souza et al., 2011; Weilhoefer et al., 2017). The species is so widespread in North America that one point an estimated 50 to 100% of invaded wetlands were dominated by PHAR (Lavergne & Molofsky, 2004; Rittenhouse, 2011). Consequently, research on the appropriate methods for PHAR control is of high importance for land managers, scientists in governmental and non-governmental/nonprofit entities, and private industry (Lavergne & Molofsky, 2004).

Among some of the most commonly used restoration methods are mechanical/manual techniques (Annen, 2008; Gerard et al., 2008; Hölzel et al., 2012; Kapust et al., 2012; Kettenring & Adams, 2011; Kolos & Banaszuk, 2013), chemical applications (Adams & Galatowitsch, 2006; Foster & Wetzel, 2005; Hayes et al., 2013; Pritekel et al., 2006; Yahnke et al., 2013), grazing treatments (Gabbard & Fowler, 2007; Hillhouse et al., 2010; Kidd & Yeakley, 2015; Law et al., 2017; Meyer et al., 2010; Sonnier et al., 2018), prescribed burning (Adams & Galatowitsch, 2006; Bowles & Jones, 2006; Foster & Wetzel, 2005; Iglay et al., 2014; Klaus & Noss, 2016; Lavergne & Molofsky, 2004; Meyer et al., 2010), and shade and exclusion treatments including solarization (Hook et al., 2009; Pfeifer-Meister et al., 2012b; Wilson et al., 2004), woody species plantings (Budny & Benscoter, 2016; Kim et al., 2006; Kuzovkina &

Quigley, 2005), and herbaceous cover crops (Iannone et al., 2008b; Perry & Galatowitsch, 2003; Perry et al., 2004, 2006).

Mixed-methods involving combinations of two or more of the former treatments are most frequently employed in wetland restoration projects targeting monotypic PHAR invasions, with burning and mowing (Foster & Wetzel, 2005; Miller-Adamany et al., 2019; Perry & Galatowitsch, 2003; Pfeifer-Meister et al., 2012a, b) and mowing/tilling with follow-up herbicide applications (Pavlegio & Kilbride, 2000; Wilson et al., 2004) most commonly utilized.

#### Mechanical Methods

Mowing is a popular restoration technique that has been frequently cited in the literature as an effective way to suppress PHAR infestations by reducing culm height and density before senescence (Gerard et al., 2008; Kapust et al., 2012; Kolos & Benaszuk, 2013; Miller-Adamany et al., 2019; Pavlegio & Kilbride, 2000; Pfeifer-Meister et al., 2012a, b). Mowing as a standalone treatment is not adequate to control PHAR's rhizomatous propagation and rigorous growth, and therefore is best applied in combination with different restoration methods within the same growing season (Gerard et al., 2008; Hook et al., 2009; Kapust et al., 2012; Pritekel et al., 2006). Effectiveness of this treatment is significantly increased when applied in combination with other restoration methods, such as herbicide application (Clark & Thomsen, 2020; Kapust et al., 2012; Pfeifer-Meister et al., 2012a, b; Pritekel et al., 2006), solarization (Johnson, 2005; Pfeifer-Meister et al., 2012a, b; Tu & Salzer, 2005) or prescribed burning (Adams & Galatowitsch, 2006; Foster & Wetzel, 2005). Repeated mowing, whereby PHAR's above-ground biomass is cut over multiple months within one growing season (annual), broadly refers to months that fall between late spring and early autumn. Some studies report mowing suppression doubles when combined with alternate restoration treatments before PHAR senesces in late July

to early October (Kolos & Benaszuk, 2013). Moreover, repeated mowing has been shown to reduce PHARs rapid early season vegetative growth in several cases where site hydrology made later season restoration treatments difficult, providing openings for native revegetation (Kolos & Benzanuk, 2013; Gerard et al., 2008). For example, one study combined repeated annual mowing over multiple years, reporting increases in native species richness of perennial graminoids and other low-lying herbaceous vegetation by the end of the time-series (Gerard et al., 2008). This experiment applied mowing to established PHAR monocultures five times over a period of five months, significantly reducing stem density in mowed plots relative to un-mowed plots (Gerard et al., 2008).

Mechanical methods are often necessary in heavily invaded sites regardless of alternate treatment planning due to PHAR's abundant litter accumulation (thatch) and belowground rhizome biomass (Miller-Adamany et al., 2019; Pavlegio & Kilbride, 2000). Sods and tillers forming PHAR's belowground biomass is usually dense enough to inhibit other taxa from establishing and can be a constant source of reinvasion if left alive (Lavergne & Molofsky, 2006). Furthermore, PHAR has the potential to re-invade a site shortly after restoration activities due to its superior vegetative structure, long-term seed viability, and advantageous response to soil disturbance and bare ground patches that often result from restoration activities (Hook et al., 2009). Research examining the changing edaphic effects on PHAR have had value in illustrating this. For example, one study experimented with sedimentation deposits of fine mason sand (a fine-textured masonry aggregate from crushed, screened, and washed quarry rock), topsoil, and control replicates (no sediment) in 140 1.1-m<sup>2</sup> mesocosms planted with native wet-prairie species, including *Carex* spp. The goal of the study was to examine the effects of three treatments (nutrients, sediment, flooding) on native species diversity and PHAR biomass over

two years (Kercher et al., 2007). Four young rhizomatous PHAR propagules (culm intact) were introduced to each experimental mesocosm and treated with one of three sediment media (none, fine mason, sand), and nutrients (lawn fertilizer) in three quantitative levels (none, low, high), at three flooding regimes corresponding to mean storm surge levels from data modelling average rainfall (in / month) in the region (constant, early season, intermittent). After two years, monitoring revealed a significant increase of PHAR in response to high, prolonged flood regimes regardless of sediment type, with absolute biomass increasing by up to twelve times greater than that of native species plantings under prolonged flooding. The native species-maintained dominance in control plots that were not subjected to any of the three treatments (nutrients, sediment, flooding), illustrating the strong influence of edaphic effects on PHAR invasiveness (Kercher et al., 2007).

Another common mechanical method used for PHAR control is tilling, also referred to as disking (Klaus & Noss, 2016; Lavergne & Molofsky, 2006; Miller-Adamanty et al., 2019; Pavlegio & Kilbride, 2000; Sonnier et al., 2018; Wilson et al., 2004). Disking is an agricultural term for ploughing that is ubiquitously used to refer to the mechanical disruption of PHAR's vegetative growth from tiller sod and rhizome bioaccumulation in restoration ecology literature. Tilling and disking methods have documented success for removing PHAR's rhizomatous and thatch biomass by manually turning surface sod layers into the parent soil, thereby uprooting underground rhizomes that are then desiccated in the mixing process (Wilson et al., 2004). Since PHAR's seed bank can remain viable for over 20 years, disking has the potential to assist in the preemptive control of dormant PHAR seeds, preventing some of the reinfestation that may occur in the absence of such treatments (Lavergne & Molofsky, 2006).

While disking is most appropriate for sites where PHAR has been established long enough to create dense mats of thatch, it can be highly destructive to the landscape, potentially resulting in unintentional negative responses from the restoration (Sonnier et al., 2018). In some cases, mechanical control methods require the use of heavy machinery, such as tractors, skid steers, mowers, and other large landscaping vehicles. As with any restoration method involving the transport of large equipment on site, transporting heavy machinery over hydric soil can be destructive. Tilling methods have the potential to disturb soil strata to the extent that extensive quantities of dormant PHAR seeds are unearthed, suppressing the regeneration of the more desirable, native species on the soil surface. Soil disturbance also alters the AMF structures and diversity in soils, potentially inhibiting native plant resurgence through the depletion of essential minerals (such as K and Fe) and altering original competition strategies between plants and soil biota (Annen, 2008; Pritekel et al., 2006).

Thick layers of thatch are prevalent in large, monotypic stands of PHAR that have established long enough to develop dense litter accumulation above the soil substrate over time and are typically dealt with before or during mowing treatments, before planting, and after herbicide application (Adams & Galatowitsch, 2006). Because of the latency with which PHAR tillers emerge from beneath thatch layers in the late winter/early spring, timing is an important consideration when applying mowing treatments. When mowing PHAR later in the season, and when the lignin of culms has hardened—having adapted to summer weather conditions by consolidating moisture into the rhizomes, it is potentially less advantageous to leave thatch on the ground. At this stage, the mulch does not readily decompose, adding to thatch biomass which can benefit early spring PHAR shoots the following season by insulating propagules from flash frost (Gerard et al., 2008).

Inadvertent nutrient deposition from the desiccation of mulch left on the ground can benefit PHAR, as decomposed organic matter may enrich topsoil ratios, prevent competing species from emerging that require higher light, and insulate PHAR propagules as they emerge the following season (Galatowitsch et al., 1999; Miller-Adamany et al., 2019). Conversely, cutting PHAR and leaving mulch on-site before flooding occurs can reduce some of PHARs competitive advantage by reducing regrowth following restoration treatments (although hydrological treatments may make native plant reestablishment challenging since extended inundation can lead to increased nutrient leaching or nutrient deposition, and alterations in soil biota) (Catford et al., 2011).

#### Herbicide

A comprehensive body of literature exists on the subject of herbicide use for PHAR control (Adams & Galatowitsch, 2006; Bahm et al., 2014; Foster & Wetzel, 2005; Hayes et al., 2013; Paveglio & Kilbride, 2000; Pfeifer-Meister et al., 2012a, b; Yahnke et al., 2013). Glyphosate is overwhelmingly represented in field-studies of herbicide use for PHAR control (Bahm et al., 2014; Bucharova & Krahulec, 2020; Clark & Thomsen, 2020; Healy & Zedler, 2010; Hovick & Reinartz, 2007; Miller-Adamany et al., 2019; Pfeifer-Meister et al., 2012a, b; Reinhardt Adams & Galatowitsch, 2005). Secondary to glyphosate in prevalence are the acetolactate synthase (ALS) inhibitor imidazoline herbicides, of which imazapic and imazapyr have received the most attention in restoration literature (Bahm & Barnes, 2011; Bahm et al., 2014; Hayes et al., 2013; Iglay et al., 2014; Kapust et al., 2012; Pritekel et al., 2006; Yahnke et al., 2013). The effectiveness of less-used herbicides, including sethoxydim (Annen, 2008; Healy & Zedler, 2010), sulfometuron methyl (Miller-Adamany et al., 2019; Pritekel et al., 2006), and atrazine (Smalling et al., 2015) have also been studied in combination with other techniques for PHAR control in North American wetlands.

The most commonly applied herbicide is glyphosate (N-[phosphonomethyl]-glycine), a broad spectrum, non-selective, systematic organophosphate herbicide with widespread use in the U.S. for agricultural, transportation, landscaping, and ecological weed control purposes (Helander et al., 2012; King & Wagner, 2010). It has remained the most widely used herbicide worldwide since the mid-20<sup>th</sup> century patenting and broad proliferation by the U.S.-based corporation Monsanto<sup>®</sup> (Helander et al., 2012). Some of its popularity was derived from successful early lab-based trials that were sponsored by Monsanto and the U.S. Department of Agriculture (USDA) throughout the latter half of the 20<sup>th</sup> century (Casler, 2010). Since the 1980s, it has had ubiquitous use in the U.S. for industrial, farming, and home, plant pest control, with some estimates of application occurring at one or multiple points in time over nearly one hundred percent (100%) of all land units (townships) in heavily converted agricultural states such as Iowa and Illinois (Helander et al., 2012).

In Washington State, many restrictions exist on the application of glyphosate near open water due to its high environmental persistence and potential to leach into drinking water sources, but it is approved for aquatic use in states that provide permitting through the EPA and Dept. of Ecology (ECY) (Yahnke, 2015). Washington State has gone through the process to approve aquatic formulations of glyphosate for use in riparian areas, although the application of non-aquatic approved formulations on nuisance vegetation in right-of-way (ROW) constitute the greatest pollution to waterbodies designated for human use (Helander et al., 2012). The potential chronic long-term and sub-lethal effects of glyphosate have received little attention throughout the history of its use, spanning over seventy (> 70) years. This was the status quo until about the

last two decades (2000 – 2020) following the release of multiple studies linking glyphosate exposure to persistent long-term health issues, and more recently, cancer (Helander et al., 2012; Zhang et al., 2019). The environmental impacts of tank mix surfactants and adjuvants have been passively examined through many chemical evaluations of primarily crop-oil based components that are commonly combined with glyphosate to aid with adherence of foliar applications to the cuticle surface of vegetation. Oil based surfactants (adjuvants) are frequently used in combination with glyphosate as a sticking agent to aid in adhering to the waxy surface characteristic of many broadleaf plants, as well as minimize off-target spray damage under high moisture/high ambient pressure conditions by decreasing the volatility of airborne droplets from pressurized spray apparatus (Yahnke et al., 2013). It is still generally assumed that tank mix formulations containing oil-based surfactants cause greater environmental harm to amphibians than glyphosate itself, though the evidence for this assumption has not been comprehensively evaluated and requires further investigation (Pfeifer-Meister et al., 2012a, b).

Several conflicting arguments exist in the literature regarding application timing of glyphosate for PHAR control in seasonal wetlands. Late winter to late spring (March – May) glyphosate applications were shown to be more effective than late summer to early fall (August – October) applications (Pfeifer-Meister et al., 2012a, b). For example, one study reported that ambient pressure and temperatures taken during a late summer application of two percent glyphosate (2%) mixed with a crop-grade, oil-based surfactant factored in to a one-hundred percent (100%) mortality rate of first-year native species plantings and zero-percent (0%) PHAR control after one growing season (Pfeifer-Meister et al., 2012a). Other studies have demonstrated effectiveness of late-season applications of glyphosate on PHAR cover, linking the rootward allocation of carbohydrates that perennial grasses undergo in the fall to treatment success (Clark
& Thomsen, 2020). Still, other reviews summarize a trend towards applying two treatments per growing season (May, September) for maximum PHAR control, citing evidence that early season applications suppress PHARs initial tillering advantage and give native vegetation a head-start, while secondary late season treatments deal substantial damage to rhizomatous biomass when PHAR is transporting complex carbohydrates below-ground in preparation for winter dormancy (Hayes et al., 2013).

In addition, other herbicides have been shown to create conditions that favor invading species by deteriorating soil quality and altering symbiotic associations between AMF, microbes, and plants (Price et al., 2018). In one study, glyphosate and sulfometuron methyl herbicide applications contributed to lasting negative effects in soil biota of several post-restoration sites that had been treated for infestations of *Cirsium arvense* (Canada thistle) and *Euphorbia esula* L. (Leafy spurge) with glyphosate, sulfometuron methyl, 2,4-D Amine (2,4-Dichlorophenoxyacetic acid), Banvel® (dicamba), Tordon® (picloram), and Plateau® (an ammonium salt of imazapic) over a four-year period (Pritekel et al., 2006). Treatment plots exhibited significantly lower microarthropod densities compared to non-treated, non-invaded plots after four years of intensive weed management, although this result may have been a consequence of bare-ground patches and soil disturbance following herbicide application rather than the chemicals themselves (Pritekel et al., 2006; Reinhardt Adams & Galatowitsch, 2005).

Glyphosate treatments have generally had favorable results on the control of PHAR when combined with mechanical methods. A study conducted in the Vancouver area of southwestern Washington State combined disking with glyphosate application in a mud-flat that had been colonized by PHAR. Compared to non-integrated pest management methods, this study found that glyphosate applied to PHAR regrowth after disking both reduced thatch biomass and

increased herbaceous species cover over bare-ground after one year of treatment (Pavlegio & Kilbride, 2000). However, the same study warned that single applications of glyphosate applied without an alternate treatment schedule resulted in zero germination of native facultative and obligate species, presumably due to a layer of dead PHAR biomass left on site after application (Pavlegio & Kilbride, 2000).

The ALS-inhibitor herbicides have been evaluated for PHAR control in several studies as secondary in prevalence to glyphosate (Bahm & Barnes, 2011; Bahm et al., 2014; Hayes et al., 2013; Iglay et al., 2014; Kapust et al., 2012; Pritekel et al., 2006; Yahnke et al., 2013, 2015). Compared with glyphosate's prevalence in restoration literature, studies on ALS-inhibitor class herbicides have limited coverage and generally are confined to field-based experiments, and in one case, a controlled, laboratory test for acute lethal exposure rates of imazapyr and imazapic formulations on amphibians (Iglay et al., 2014; Hayes et al., 2013; Yahnke et al., 2013). Imazapyr (common trade names include Habitat® and Polaris®) is a non-selective ALS-inhibitor herbicide that can be applied either as a pre-emergent herbicide or as foliar treatment on PHAR vegetative growth (Hayes et al., 2013; Yahnke et al., 2013). Imazapyr has a ninety-six hour (96h) median lethal concentration of over one hundred milligrams per liter (LC50 > 100 mg/L) for aquatic invertebrates and fish, and a low (4.6-5.0 ppm) to high (9.5-9.7 ppm) range for impacts to amphibians (Yahnke et al., 2013). A gap in research exists for comprehensive imazapyr use in aquatic environments, including published literature on its effects on PHAR. Imazapyr's toxicity on amphibians was evaluated in a study that found acute-lethal levels specified by the product label had no observable effects on amphibians (Yahnke, 2015). Owing to imazapyr's somewhat brief solubility extent in water (3-5 days) and presumably lower detriment to aquatic wildlife

than glyphosate, further investigation on this herbicide could be beneficial for PHAR control efforts and OSF conservation.

Finally, grass-specific herbicides are intermittently discussed in the literature and warrant a brief overview. The graminicide sethoxydim (Vantage®) applied to early-season PHAR tillers and seedling cover has been evaluated in several studies (Annen, 2008; Healy & Zedler, 2010). In one study, a sethoxydim and tillage combination was more effective on post-emergence PHAR cover than herbicide alone or in combination with other plant growth regulator treatments (Cycocel®/Proxy®) (Annen, 2008). Additionally, a water-conditioning agent was added to sethoxydim tank mixes in this study to accelerate some of the residual effects of the chemical half-life after application (Annen, 2008). However, this herbicide is not approved for direct water application due to the toxicity of its solvent naphthalene, so its use in Washington State is limited to temporal wetlands during dry seasons (Healy & Zedler, 2010). Furthermore, sethoxydim can prevent native seed recruitment on account of its residual persistence and mode of action, as evidenced by results in a study showing a complete reverse in control of PHAR cover by the third year of treatments (Healy & Zedler, 2010).

### **Prescribed Burning**

Control of PHAR by burning has been evaluated in several studies (Bowles & Jones, 2006; Foster & Wetzel, 2004). Prescribed fire treatments are primarily conducted in open-canopy riparian habitats that have minimal fuel sources, such as grassland or prairie wetlands. Many landscapes in the Great Plains as well as in the Pacific Northwest were burned for thousands of years by some Native American tribes on their land (Storm & Shebitz, 2006). Thus, prescribed burning is both historically and contemporarily relevant to the landscape in many parts of the U.S. and is perceived as beneficial for restoration purposes for several reasons. Burning allegedly

encourages floristic beta-diversity and abundance in microarthropod communities while supporting specialist species in cohabitation with the more common, generalist species that often dominate wetland habitats (Kettenring & Adams, 2011; Klaus & Noss, 2016; Meyer et al., 2010). Burning can also flush out the invasive seed bank, causing many dormant seeds to germinate at once in the following growing season which has benefits for long-term revegetation efforts (Bucharova & Krahulec, 2020).

Later trials with fire and herbicide treatments (with glyphosate) were ineffective at controlling PHAR over the long-term in several cases (Healy & Zedler, 2010). Many studies initially reported reductions in PHAR cover at or greater than fifty percent ( $\geq$  50%), but many also reported a trend of reinvasion within one to two years after application (Healy & Zedler, 2010; Maurer & Zedler, 2002). One study found significantly reduced PHAR seed-bank density and living biomass (*F* = 131.34, *p* < 0.01) one year following spring burn and glyphosate application (Adams & Galatowitsch, 2006).

The environmental conditions that accompany seasonally inundated wetlands, including periodic hydrological depths, and thus the potential of continual exposure to PHAR seed-drift, discourage repeated soil exposure and bare-ground, possibly precluding the chance of competitive germination by native species when the seedbank is pervaded by PHAR (Reinhardt Adams & Galatowitsch, 2005; Foster & Wetzel 2005; Meyer et al., 2010). Another possible explanation for the low success of burn treatments could be oversight of PHAR's below-ground biomass. Shallow, quick burns that only immolate the first one to two inches of soil organic matter can stimulate the seed bank, especially if burning is followed up by tilling. PHAR can rebound the following season after the initial treatment if below-ground rhizomes are not completely removed or killed. Thus, in PHAR's case, burning treatments would have to be

extremely intensive, reaching temperatures conducive for ground fires that are far beyond what is either permissible or advisable for prescribed burning (Foster & Wetzel, 2005).

### Grazing

Grazing is a well-documented topic in the literature, both as a standalone treatment, or in combination with other restoration methods for its compatibility in sites already exposed to agricultural use (Meyer et al., 2010). The use of livestock for PHAR control has been evaluated in several studies as a chemical-free alternative, or as regimented treatment regime applied in combination with other restoration techniques (Burmeier et al., 2011; Hillhouse et al., 2010; Kidd & Yeakley, 2015; Larson, 2014; Sonnier et al., 2018). Common applications of this method include rotational grazing, livestock exclusion followed by replanting and intensive grazing over large areas for extended periods of time (Kidd & Yeakley, 2015; Sonnier et al., 2018).

Cattle grazing has historical prevalence in the Lower Puget Sound region of Washington State, where PHAR was intentionally planted for use as a forage crop due to its value as yeararound feeding stock for livestock (Kidd & Yeakly, 2015). The effectiveness of livestock grazing on PHAR cover has produced mixed results. Perhaps because of the difficulty of measuring grazing effectiveness in restorations combined with multiple other treatment types, some studies have reported little or no effect of grazing on native vegetation composition and cover and/or PHAR cover (Hillhouse et al., 2010; Sonnier et al., 2018). Other studies have found grazing to be quite effective in specific situations, such as agricultural wetland sites and floodplains exposed to intensive, regimented grazing over several years (Burmeier et al., 2011; Larson, 2014).

Additionally, some of the disadvantages of utilizing grazing treatments have been documented in the literature. For example, the side effect of bare soil exposed from excessive grazing has been implicated in reduced ecological functioning and increased PHAR invasion

(Gabbard & Fowler, 2007; Hillhouse et al., 2010; Matthews et al., 2020). Livestock traffic can wear and tear on soil and vegetation, negating the positive effects of invasive cover reduction from herbivores by decreasing survival and cover area of native species, opening bare ground patches, and disturbing dormant seeds in the soil (Larson 2014).

Grazing has documented application in PHAR-dominated wetland restorations. In a case study on rotational grazing, effects of this treatment were observed on native species richness and PHAR abundance over time in cattle grazed plots that were seasonally rotated in correspondence to the germination timing and maturity periods of native vegetation (Hillhouse et al., 2010). After two years of rotational grazing, thatch biomass was reduced by about seven percent (7.3%) in grazed plots, and bare ground cover increased by eleven percent (11%) in grazed plots. These changes were not apparent until the second year of restoration treatments after native species richness and relative basal PHAR cover had been observed to increase and decrease, respectively. Minimal gains from the first two years of rotational grazing were outpaced by PHAR by year three from the cumulative effects of soil disturbance, incidental seed dispersal, and bare ground patches that opened space for reinvasion, ultimately superseding the positive effects of the grazing treatments in favor of the invader (Hillhouse et al., 2010).

Grazing can also occur by wild animals, though PHAR's high phenolic alkaloid content makes it unpalatable to most herbivores. Grazing by wild ungulates such as *Odocoileus virginianus* (white-tailed deer) and *Cervus canadensis roosevelti* (Roosevelt elk) is known to occur but does not exert significant pressure on PHAR cover in the Pacific Northwest (Kratzer, 2014). Herbivory of PHAR shoots, stems, and leaves by *Castor canadensis* (North American beaver) also occurs naturally but is often discouraged by some organizations because of the potential for hydrological and soil disturbance to spread invasion (Law et al., 2017; Matthews et

al., 2020). Substantial evidence exists to suggest beaver are essential ecosystem engineers that, when allowed to modify hydrology in their natural habitat, sustain wetland the functions of critical habitat for declining species. Beaver activities help sustain amphibian populations by causing great hydrological changes to downstream landscapes. These activities promote stable hydric states conducive to specialist species such as the OSF by producing hydric conditions that favor emergent vegetation and the occurrence of hydroperiods that form the unique spatial heterogeneity characteristic of OSF habitat, with all its furrows and hammocks, shallow pools, and other varied hydrological features (McAllister & White, 2001). Further, the natural regime shifts caused by beaver have been shown to support the OSF overwintering habitat requirements by aiding in the formation of deep pools proximate to oviposition habitat that are connected by hydrology (Hayes et al., 2001). Thus, part of the attractiveness of herbivory, whether caused by domesticated ruminants or wildlife, lie in its potential to counterbalance the displacement of natural hydrological regimes that have resulted from human intervention (i.e., beaver trapping and dam destruction, river and tributary damming and modification) (Schooler et al., 2009).

# Solarization

Limited documentation exists of invasive species control with solarization methods compared to other techniques examined in this literature review, although it has retained comparatively lesser prevalence for several decades (Lavergne & Molofsky, 2006; Pfeifer-Meister et al., 2012b). Solarization refers to the use of synthetic or natural material coverings for invasive species control, usually accomplished with plastic sheeting. The invasive vegetation is covered by solarization plastic, and then left to desiccate for a determinate time period (min. 2 weeks, max 2 years) while exposed to intense heat from solar radiation amplified underneath the plastic, gradually killing rhizomes from these effects combined with sustained photosynthesis deprivation (Gerard et al., 2008; Hook et al., 2009; Johnson, 2005; Lavergne & Molofsky, 2006; Maurer & Zedler, 2002; Pfeifer-Meister et al., 2012b; Tu & Salzer, 2005; Wilson et al., 2004). Dark colored plastic coverings are also employed at times as darker colors are known to absorb heat, although lighter colors are more commonly employed such as white-opaque (amplifies solar radiation), and occasionally solid white (Pfeifer-Meister et al., 2012b).

Solarization treatments have had mixed results in the control of PHAR, showing some measured effects of desiccation beneath localized treatment plots receiving sustained treatments (Pfeifer-Meister et al., 2012b). In one study, solarization reduced native species diversity and richness over three years, establishing favorable soil conditions for the germination of early native perennial grasses at a rate nearly three times greater than that of the other treatments in the study (herbicide, mechanical) (Pfeifer-Meister et al., 2012b). While the thermal requirements for rhizomatous desiccation are low for most species, some studies report that a minimum of two years is necessary to control PHAR using solarization plastic (Laverge & Molofsky, 2006). Ecosystem respiration, above-ground net primary productivity, AMF colonization, and total native herbaceous plant cover was up to two times higher in solarization treatment plots than topsoil removal plots (Pfeifer-Meister et al., 2012a, b). However, it should be noted that the occurrence of microbial mortality and abiotic depletion in topsoil subjected to solarization under high temperatures can easily impede native revegetation efforts.

#### **Biological Control**

Extraordinarily little discussion exists of biological control methods for PHAR in the literature since few, if any, reliable agents have been identified to date. In some areas of the conterminous U.S. biocontrol methods for PHAR are restricted due to its economic value for forage cropping and biofuel production (Healy & Zedler, 2005). Ultimately, biological methods

have been deemed unreliable for PHAR control by reason of considerably less thoroughly evaluated predictors existing for their success compared to other methods. Most notably, the increase of indole alkaloid that PHAR produces when exposed to herbivory is a useful adaptation that, while more pronounced in some cultivars than others, has precluded most biocontrol efforts (Hayes et al., 2013). Biological control agents for PHAR are significantly deterred by the high alkaloid levels in North American cultivars—hormones some cultivars have selected as part of an exceedingly effective suite of defensive mechanisms against predation (Hayes et al., 2013). This survival mechanism was observed in the late 1970s by Byers and Sherwood (1979) while studying the effects of the biological control agent *Oscinella frit* (Frit fly) on PHARs aboveground biomass (Hayes et al., 2013). Herbivory deterrence by PHARs phenolic content has been reported in wild ungulate herbivores, though not for beaver (Kratzer, 2014).

#### Hydrological Restoration

Both the hydrological components of wetlands as well as those related to restoration objectives themselves have wide representation in the literature (Cusell et al., 2015; Green & Galatowitsch, 2001; Jakubowski et al., 2010; Kercher et al., 2007; Lavergne & Molofsky, 2006; Magee & Kentula, 2005; Maurer et al., 2003; Meyer et al., 2010; Sonnier et al., 2018). In an ecological sense, hydrology is often evaluated as an indicator of wetland health, vegetation composition, and restoration potential. In restoration methodology, hydrological restoration refers to the intentional manipulation of water functions either to mimic the hydroperiods of reference wetlands or create different hydrological regimes in support of restoration objectives (Gerard et al., 2008; Magee & Kentula, 2005; Weilhoefer et al., 2017). Hydrological restoration may occur through natural regime alterations, or human-induced flow control in irrigated or dammed systems, or by use of sedimentation deposition or control (Magee & Kentula, 2005; Matthews et al., 2020; Sonnier et al., 2018), and mechanical applications, which may entail mulching, haying and tilling (Clark & Thomsen, 2020; Miller-Adamany et al., 2019), or native seeding, bareroot planting, or transplanting (Hölzel et al., 2012; Lindig-Cisneros & Zedler, 2002; Miller-Adamany et al., 2019; Pritekel et al., 2006; Wilson et al., 2004). Additionally, some wetland restorations have involved hydrological manipulation as a form of invasive species control or as a mechanism to aid in native vegetation establishment during or after restoration activities take place (Cusell et al., 2015; Iannone et al., 2008b; Kercher et al., 2007).

Human-caused flooding is a form of hydrological restoration used for wetlands with modified hydroperiods from damming, agricultural water diversion, or prolonged drought from climate change. Where needed, the use of temporary flooding treatments can promote herbaceous plant assemblages associated with the spatial variation of remnant wetlands, such as the presence of topographic heterogeneity (i.e., troughs, shallows, and hummocks) characteristic of riparian landscapes with varying hydrological activity and dominant plant communities of cespitose, bunching, emergent vegetation (Pfeifer-Meister et al., 2012a). This should not be confused with sustained inundation (which is also a form of hydrological restoration for different wetland types), which is not conducive to the OSF life cycle. As discussed before, these are some of the most important habitat characteristics for the OSF. However, in some cases the absence of ecological pressures can facilitate paradigm shifts in plant community structure or composition on a watershed-scale. One such transition was attributed to a rapid ecological shift from primarily native hydrophytic vegetation assemblages to prevailing monotypic PHAR stands within two growing seasons (Catford et al., 2011).

Water depth is an important component in the post-restoration success of native planting survival, as well as the persistence of invasive species (Magee & Kentula, 2005; Pfeifer-Meister

et al., 2012b). In wetland areas located downstream from dams, for example, hydrological manipulation has shown demonstrated effectiveness at establishing hydrophytic vegetation through the restoration of historical hydroperiods and summer dry periods (Weilhoefer et al., 2017). Some studies have found that intermediate hydrology zones favored greater native-species colonization after restoration. In contrast, one study found that intermediate-level hydrology seemed to enable PHAR reinvasion after restoration, which, after hydrology had been altered, easily outcompeted native vegetation in intermediate hydrology levels within ten centimeters ( $\leq$  10cm) of variability (Magee & Kentula, 2005).

Hydrological restoration can also serve as a pre-treatment regime in highly degraded wetlands, facilitating native planting survival. In many cases, hydrological restoration is required for invasive species control methods to be implemented effectively, and to ensure the potential survival of any native revegetation measures. This is especially the case in wetlands that are connected by larger riparian systems, where the seed bank is continually replenished by upstream PHAR infestations after the occurrence of peak seasonal flows (Matthews et al., 2020; Maurer et al., 2003; Miller-Adamany et al., 2019).

# **Revegetation & Native Species Selection**

Substantial material exists in the literature that focuses on the importance of establishing native vegetation at some point in the restoration process to control PHAR over the long-term (Iannone et al., 2008b; Ormshaw & Duval, 2020; Taddeo & Dronova, 2018). The primary goal of cultural restoration is to counter the invasive mechanisms of the invading target species with competitive advantages inherent to the native species or legacy-site community, wetland system, and functional attributes of the target site. A few examples of common revegetation methods include direct seeding by hand or with the aid of mechanical spreaders and other equipment

(Applestein et al., 2018; Bucharova & Krahulec, 2020; Kettenring & Tarsa, 2020; Lavergne & Molofsky, 2006; Matthews et al., 2020; Perry & Galatowitsch, 2003). When possible, culturalmechanical treatments of PHAR are frequently used together to facilitate native vegetation regeneration in tandem with invasive species control to reduce the impact of soil disturbance caused by restoration activities. Cultural revegetation restoration techniques have been combined with mechanical treatments in some studies, including topsoil tilling, mulching, or haying to stimulate the native and invasive seed bank or enrich the topsoil with amendments (Applestein et al., 2018; Miller-Adamany et al., 2019). Potted and bareroot plantings, plugs, or broadcast seeding methods are most commonly used to reintroduce native plants (Matthews et al., 2020; Miller-Adamany et al., 2019).

Seeding native cover crops in restored agricultural areas has shown mixed success on PHAR control and suppression (Maurer & Zedler, 2002; Perry & Galatowitsch, 2006). Broadcast seeding of cover crops may be more effective in ephemeral wetlands that are not connected to larger wetland systems due to the risk of reinvasion from flooding (Healy & Zedler, 2010). Sowing graminoid and forb seed has repeatedly failed to preempt PHAR's early spring/late winter tillering from axial rhizome buds (Healy & Zedler, 2010). In one case, epinastic PHAR regrowth that previously had been treated with glyphosate reduced light availability for native herbaceous plants by eighty-five percent (85%) when PHAR culms had attained less than one meter (< 1 m) of vegetative height (Healy & Zedler, 2010).

Native species reintroductions, spread across a wide range of subtopics within wetland restoration and ecology, is well established in the literature (Quesnelle et al., 2013). Hydrological levels, hydric soil types, and hydroperiods often determine the survival of native plantings, so before restoration activities take place management objectives should consider the suitability

native planting selections in respect to the natural hydrological regimes of the restoration site (Mulhouse & Galatowitsch, 2002). Ecological responses observed from prescribed restoration methods, selected native species plantings, and treatment timelines are considerations worth noting as well. Vegetation survival in the first few years after restoration can be volatile as multiple stressors presented by the disturbances of restoration activities may exceed the capacity of plantings to compete with invasive seed bank propagation (Adams & Galatowitsch, 2006; Wilson et al., 2004).

Besides anthropogenic and restoration stressors on a given study area, a variety of adverse reactions in native plant taxa to the soil biota become evident only after project deployment has occurred and native species revegetation has been attempted (Magee & Kentula, 2005). One way this issue can manifest is when biotic interactions between microorganisms and their food sources create significant changes in soil chemistry post-restoration—an ancillary result of soil conditions present before restoration, or those caused by the restoration techniques themselves (i.e., by disturbing soil, adding soil substrate or other amendments, or introducing an unfamiliar species that dominate mycorrhizal conditions on a significant level) (Weilhoefer et al., 2017). Additionally, altering topsoil conditions over any portion of the landscape can reduce transplant survival, increase the likelihood of seedling consumption by herbivores, and reopen vulnerabilities to reinvasion by PHAR or other exotic species (Kneller et al., 2018). This can affect restoration efforts both financially and logistically, since established monocultures of PHAR in seasonally flooded wetlands require annual retreatments and monitoring to maintain progress (Bahm et al., 2014).

Poor recruitment and survival of native plantings is sometimes linked to the residual effects of BH by invasive species, as well as the proximity of the restoration site to neighboring

invaded areas. The long-term effects of BH are often observed in the reduced performance of native species to establish dominance ecological functions, and biological shifts tipped by climactic effects, including alterations in soil moisture content, chemical structure, and biotic assemblages (Reed et al., 2019).

Challenges that often arise in the revegetation process include high rates of post-planting mortality that occurs when dormancy periods of the native planting stock are not coordinated with the timing of installation in the field, or in the advent of abnormal winter weather phenomena (Kettenring & Tarsa, 2020). This can occur when knowledge of the natural history of the site is hard to obtain or not fully evaluated before revegetation orders are processed. As a result, additional planting prescriptions beyond the extent of initial plant orders can increase costs of the revegetation plan, which may be exacerbated by other effects of the restoration project, such as increased bare-ground patches and low rates of native species recruitment and survival (Bahm et al., 2014; Bennett et al., 2011; Kettenring & Adams, 2011). Additionally, restored sites that do not recover appropriately following restoration activities may have: 1) incurred excess disturbance from weed management activities without amelioration, 2) not received post-restoration re-treatments or monitoring to contain invasive regrowth, or 3) not been revegetated after weed control activities (Bucharova & Krahulec, 2020). For example, in an extensive, nine-year evaluation of restoration outcomes in 41 PHAR-dominated wetland sites, PHAR increased by sixty to one hundred percent (60 - 100%) in the restorations, comprising the dominant species in 39 out of 41 total restored wetlands by the ninth year of the time series (Mulhouse & Galatowitsch, 2002). These findings shed significant doubt on whether PHAR control efforts were effective without follow up weed control measures, irrespective of hydrological or restoration outcomes (Mulhouse & Galatowitsch, 2002).

Erosion is also a frequent problem after restoration has been conducted, particularly following broadcast herbicide treatments that are applied to infestations along lacustric margins of waterbodies (Kneller et al., 2018). Even in situations where preemptive bank re-stabilization is completed before restoration occurs, it may be necessary to consider post-restoration fill-in and soil substrate supplements or additives since PHAR contributes to high vegetation-channel roughness (referring to the erosion rate /slope along the vegetation margin) and provides insufficient surge protection, which can lead to bank erosion (Martinez & McDowell, 2016). Bank stabilization can be achieved relatively cheaply with the use of coir bioswales, burlap blankets pinned to shorelines, and live staking with native woody species that have high auxin and clonal propagation (such as *Salix* spp.) (Hook et al., 2009; Kim et al., 2006; Kuzovkina & Quigley, 2005).

Effective native plant revegetation projects are generally tailored to fit site-specific characteristics, including local climate, hydrology, topography, and accessibility considerations. For example, topsoil amendments are generally applied after site preparation and invasive species control activities, as haying and mulching treatments are applied after both control and revegetation measures are accomplished. The repurposing of cut vegetation from mowing as mulching byproduct is both time and cost efficient. It also aids in the establishment of plantings by providing frost cover, suppression of invasive regrowth from seed or tillers, and supplements plantings with some additional nutrients over time as amendments decompose (Kneller et al., 2018).

Revegetation by seeding is well documented for sites where native graminoid species are adapted to edaphic and hydrology characteristics, such as soil pH (alkalinity-acidity) and hydroperiods (Pfeifer-Meister et al., 2012b). Direct seeding can be accomplished in a variety of

ways, including mechanical spreading with rototillers, or direct seeding by hand (Kneller, et al., 2018). Thatch removal is often required immediately after restoration if was not accomplished by the restoration methods used. It provides young plantings with adequate access to sunlight and soil contact during the first few months of revegetation (Hölzel et al., 2012; Pfeifer-Meister et al., 2012b). Bareroot plantings, seedling plugs, and potted plantings constitute most revegetation orders (Miller-Adamany et al., 2019). These types of plantings have higher survival rates over time and are more likely to be able to withstand periodic inundation and flooding events than younger propagules from plugs or direct seeding applications. Depending on the selected taxa, mature plantings can be more costly than broadcast seeding, often require intensive labor in comparison, and may pose logistical constraints to project maintenance feasibility in remote areas (Miller-Adamany et al., 2019).

Not all native planting projects are successful, and many fail for diverse reasons that range from poor soil conditions, variable hydrology and native species selection, to complications from the residual effects of restoration treatments themselves (Pfeifer-Meister et al., 2012a, b). In general, native species tend to have more difficulty establishing than exotic/invasive species in their natural range after long-term invasion, and depending on the method of planting during restoration, may need additional assistance to survive the first few years after planting (Martinez & McDowell, 2016).

Finally, both revegetation and PHAR control efforts have had documented improvements from management plans that included cultural restoration methods involving the addition of topsoil nutrients and other soil amendments. Nitrate addition in prairie-potholes and N-poor conditions for PHAR control have shown some promise, though still represent an area that deserves more attention in the literature (Perry & Galatowitsch, 2004).

## **Other Shade Treatments**

Some of PHAR's spatial monopolization can be counteracted with densely packed plantings containing primarily woody species-perceivably warranting shorter investments of time, money, and other resources (Budny & Benscoter, 2016; Hovick & Reinhardt, 2007; Kim et al., 2006; Maurer & Zedler, 2002). Woody species establishment has attained widespread acceptance in restoration ecology as a shading and exclusion mechanism against PHAR's aggressive rhizomatous spread, and is almost exclusively deployed in wetland mitigation in Washington State (Adusumilli, 2015; Budny & Benscoter, 2016; Bendor, 2009; Bendor & Riggsbee, 2011). The method is attractive for its effectiveness at establishing full canopy cover within narrow compensatory wetland mitigation timelines, or under limited restoration budgets for ecological projects. Popular species selections in the Pacific Northwest are *Salix* spp. (Willow) and *Cornus sericea* (Red-osier dogwood). Revegetation with *Salix* spp. and other select woody species have also exhibited reliable long-term PHAR control in several studies (Hovick & Reinhardt, 2007; Miller-Adamany et al., 2019). These species are often chosen because they readily establish from live cuttings ('stakes') in most hydric soils, potentially outpacing PHAR's vegetative height within a few years with a low initial cost and minimal oversight (Kim et al., 2006; Kuzovkina & Quigley, 2005).

Proponents of woody planting canopy closures have often tried to rationalize its broadscale use in any restoration project seeking to suppress or eliminate PHAR monocultures in wetlands, but significant information also exists in the literature that suggests it may not always be successful (Holland et al., 1995; Kim et al., 2006; Kuzovkina & Quigley, 2005; Ormshaw & Duval, 2020). As a species that depends on mutual symbiosis with AMF, *Salix* can fail to establish in soils that have been homogenized by PHAR over a long period of time due to the

residual biotic and abiotic soil changes that such infestations can cause. Also, *Salix* spp. are sensitive to high water-soil saturation levels, making restoration of this species a gamble under waterlogged, PHAR dominated conditions (Kuzovkina & Quigley, 2005; Ormshaw & Duval, 2020). Furthermore, young plantings or live cuttings can easily become overcrowded in the early spring, when PHARs aggressive above-ground growth rate is at its highest (Wilson et al., 2004).

Finally, *Salix* spp. accommodate high water transpiration loss in wetlands: a process that is capable of significantly altering wetland functioning over the long-term (Budny & Benscoter, 2016). A combination of *Salix* spp. leaf area index (LAI) and high stomatal conductance give way to PHARs high carbon fixation rates and heat resistance. The ratios of carbon fixation can be offset by evapotranspiration rates, which can lead to topsoil deterioration, nutrient leaching, and erosion (Budny & Benscoter, 2016). Thus, it is recommended to avoid encouraging the monotypic establishment of *Salix* spp. in PHAR-dominated areas due to the risk of transpiration loss occurring to the extent that wetland succession is prematurely catalyzed (Budny & Benscoter, 2016; Kuzovkina & Quigley, 2005).

Plant canopy shading can also be accomplished with herbaceous forbs and graminoids through the broadcast seeding of cover crops, though due to PHAR's tall vegetative height very few herbaceous species can attain greater vertical stature. Accordingly, reference to shade treatments in restoration literature usually relates to the technique of using shrub, tree, and other woody species plantings to attain full canopy closure (Maurer & Zedler, 2002). As discussed earlier, woody species establishment is not recommended for OSF habitat restorations because closed canopies can exclude oviposition and rearing habits entirely.

#### **Amphibian Responses to Restoration Methods**

So far this literature review has identified some of the most common restoration methods employed for PHAR control in palustrine-emergent, ephemeral wetlands, and wet prairies. We have seen how restoration activities can sometimes have negative impacts on the floral and faunal components of restored wetlands both during and shortly after invasive species control has occurred. Since invasive species control efforts often are associated with management objectives relating to the restoration of native fauna as well, it is fortuitous to examine the potential interactions between restoration treatments and wildlife species of concern. Additionally, since amphibian declines are occurring more rapidly in the 21<sup>st</sup> century than ever before—an effect compounded by their susceptibility to environmental changes and the lack of comprehensive federal standards for the conservation for many species—restoration techniques that favor specialist species have substantially increased in recent years (Horvath et al., 2017; Matthews et al., 2020).

Sufficient information exists in the literature regarding the effects of restoration treatments on amphibian populations, including the morphological responses and latent effects of reduced species diversity from restoration activities (Battaglin et al., 2016; Brown et al., 2012; Cook & Hayes, 2020; Hua et al., 2013; Iglay et al., 2014; King & Wagner, 2010; Klaus & Noss, 2016; Krueger et al., 2017; Mester et al., 2020; Rowe & Garcia, 2014; Smalling et al., 2013; Yahnke et al., 2013). As discussed earlier, one of the strongest indicators of regional biodiversity and ecosystem health can be measured through the response of herpetofauna to human-related impacts, specifically those incurred by land use management and restoration (Brown et al., 2012; Dudgeon et al., 2006; Holgerson et al., 2019; King & Wagner, 2010; McKibbin et al., 2008; Reeves et al., 2016; Stuart et al., 2003).

Herbicide effects on amphibians have been evaluated the most out of any restoration treatments. Some accumulations of more persistent herbicide types found in amphibian tissues from prolonged or high-level chemical exposure have been inexorably linked to their decline. Except for glyphosate, those most frequently implicated have a higher order of magnitude of residual environmental persistence and toxicity to amphibians than those that degrade more quickly (Battaglin et al., 2016; Hua et al., 2013; Smalling et al., 2015).

Some attention has been given to the acute and sub-lethal effects of aquatically approved glyphosate applications in wildlife and plants in controlled experiments (Bahm & Barnes, 2011; Bahm et al., 2014; Yahnke et al., 2013). While glyphosate has historically been labelled as non-toxic to aquatic life, aquatic approved formulations of glyphosate in Washington State have exhibited both sublethal and acute toxicity in amphibians, warranting precautions to open-water contamination and off-target damage to native vegetation in wetland areas (Hayes et al., 2013; King & Wagner, 2010; Yahnke et al., 2013).

Amphibians in the northwestern U.S. have received considerable attention in the literature for exhibiting symptoms of acute and sub-lethal toxicity to glyphosate herbicides approved for professional and public use, including aquatically approved formulations and their common crop-oil based surfactants (Battaglin et al., 2016; Helander et al., 2012; Iglay et al., 2014; King & Wagner, 2010). The response of amphibians to glyphosate is regarded as two-pronged, with one exposure vector occurring from direct exposure to the herbicide in water, and the other occurring over time from the residual effects of its environmental persistence (Hua et al., 2013; King & Wagner, 2010). Sublethal, long-term effects of glyphosate and its common surfactant formulations manifest in amphibians as birth complications, including deformities,

pre-metamorphic mortality, and physiological differences in hatched larvae, such as smaller S:V (Snout-Vent) lengths (King & Wagner, 2010; Yahnke, 2015).

Glyphosate toxicity in lentic-breeding amphibians appears to be consistent across scales of potency. One laboratory aquaria experiment tested the toxic effects of around fifty percent active ingredient glyphosate concentration per liter of water (Roundup® Regular: 50.2% mg AI/L) on six native Pacific Northwest amphibians: Long-toed salamander (*Ambystoma macrodactylum*), Northwestern salamander (*Ambystoma gracile*), Western toad (*Anaxyrus* [*Bufo*] boreas), Pacific tree frog (*Pseudacris regilla*), Cascades frog (*Rana cascadae*) and Columbia spotted frog (*Rana luteiventris*) (King & Wagner, 2010). Results showed that following the twenty-four-hour (24-h) mark of pond breeding amphibian embryo and larvae exposure to acute lethal concentrations (LC50) of glyphosate, all anuran species samples had expired. Moreover, all amphibians studied, including salamanders, showed sensitivity to low LC50<sub>24-h</sub> concentrations of glyphosate and surfactant tank mix exposures (King & Wagner, 2010). The results of this experiment revealed how even sub-lethal levels of glyphosate can pose a threat to amphibians in a laboratory setting. How such effects of glyphosate impact lenticdwelling amphibian species in their natural habitat remains unclear.

One emerging topic in restoration science deals with the effects of prescribed fire and changing climate conditions on amphibians (Cook & Hayes, 2020; Iglay et al., 2014; Reinhardt Adams & Galatowitsch, 2005). The effects of fire on frogs have limited research cover but have been increasing in frequency for the past decade. More evidence appears to exist for the beneficial aspects of prescribed fire on amphibians than otherwise. One recent study found that out of four amphibian species present before prescribed burning, all were present the following year as well as an additional native anuran not previously known to inhabit the site (Cook &

Hayes, 2020). Increasing occurrences of fires due to climate change effects, including longer warm seasons and reduced precipitation in some areas of the Pacific Northwest, warrant further investigation into the impact these patterns will have on already imperiled amphibian populations (Cook & Hayes, 2020). The relationship between precipitation, wetland hydrology and OSF mortality are most evident in early offspring, such as in the case of tadpoles that become stranded in dried shallows following the onset of high temperatures and receding hydrology (Hayes et al., 2006). While burn events in hydrologically shallow, ephemeral wetlands can temporarily reduce the water levels required for specialist amphibian oviposition in shallow pools, burning has also been shown to maintain habitat features important to anurans such as the OSF (Cook & Hayes, 2020).

Finally, mechanical restoration methods have been shown to have positive effects on amphibian breeding habitat in some cases. Mowing treatments can increase OSF oviposition habitat in wetlands lacking in canopy openness by reducing vegetation height around shallow pools to encourage egg-mass deposition (Mester et al., 2020). However, mowing can have the opposite effect on larval survival when oviposition timing is late, heat waves cause rapid evaporation of shallow water sources, or hydrology is already low for sites that have been physically altered. In this case, mowing tall vegetation can cause water temperature to rise, affecting both the oviposition choices of frogs and the survival of offspring (Kapust et al., 2012; Rittenhouse, 2011). For the OSF, which is a warm water-adapted species, this can be beneficial for survival.

# **Alternative Methods**

As identified in this literature review, many ways exist for restoration actions to fail to suppress or control PHAR once it has become established, something especially evident in

restoration sites connected by hydrology (Galatowitsch et al., 1999; Hayes et al., 2013; Perry et al., 2004). The competitive mechanisms of PHAR enable it to rapidly adapt to most ecological changes, including those incurred intentionally for its control (Reinhardt Adams & Galatowitsch, 2005). Consequently, some ecologists have called for more informed, intensive approaches to PHAR control that combine traditional methods with cultural methods, make use of interdisciplinary approaches, or consider locally relevant information about hydrology, natural history, climate change, and genomics-based evidence for PHARs potential adaptations to the proposed restoration (Hook & Klausmann, 2006; Matthews et al., 2020; Tanaka et al., 2008; Thom et al., 2004).

Cultural restoration methods that combine revegetation strategies with nutrient addition, soil plant material transfer, or biodegradable material application techniques have limited prevalence in the literature but have recently gained popularity in tandem with the surge of green-energy and bioengineering research since the mid-2010s (Banerjee & Krahulec, 2000; Daria et al., 2020). Some examples of cultural and bioengineering methods include topsoil amendments (Kneller et al., 2018), pre-vegetated soil-strips (Matthews et al., 2020), plant mat transfers (within-site) (Sparks et al., 2013), pre-vegetated, natural fiber mats (Hook & Klausmann, 2006; Hook et al., 2009), and other biodegradable geotextile ground coverings (Burmeier et al., 2011; Caffrey et al., 2010; Daria et al., 2020; Hoffmann et al., 2013; Maiti & Maiti, 2015). Soil amendments may not give native plantings an advantage over invasive plants and may not be as beneficial as the pre-existing topsoil on site. Also, micro-invertebrate activity may be stimulated by soil amendments such as compost or mulch that is incorporated into the soil, which can be problematic for native plantings, as new cotyledons are particularly vulnerable to herbivory from microarthropods (Kneller et al., 2018).

Use of pre-vegetated mats from natural fibers, most commonly coconut coir-pith and coir-dust (Tanaka et al., 2008; Vishnudas et al., 2006), jute (Caffrey et al., 2010), and biodegradable synthetic materials (including composites) are well documented in bioengineering and erosion control research, but poorly evaluated for ecological use in restoration literature (Hoffman et al., 2013; Hook & Klausmann, 2006; Hook et al., 2009). Pre-vegetated coconut-coir mats have been examined in a variety of studies for their effect at establishing vegetation on eroding slopes (Boustany, 2004; Maiti & Maiti, 2015; Tanaka et al., 2008; Vishnudas et al., 2006) but have extremely limited coverage in wetland restoration literature with well-defined ecological objectives (Hoffmann et al., 2013; Hook & Klausmann, 2006; Hook et al., 2009). The application of natural fiber substrates in engineering and stormwater contexts have continually exhibited positive outcomes in native vegetation establishment and hydrological remediation, indicating their ability to provide the dual benefits of soil stabilization and nutrient enrichment from biodegradation over time. Thus, with the increasing popularity of geotextiles for erosion control and wetland revegetation in recent years, additional research is needed to comprehensively evaluate ecological applications of pre-vegetated natural fiber products in restoration contexts.

Natural geotextiles have been utilized in slope erosion design concepts and engineering since at least the early 1980s, but their development dates as far back as the 1960s in forage cropping (Allen & Leech, 1997). Perhaps some of the earliest detailed accounts of coconut-coir uses for bank stabilization were a series of reports released by the U.S. Army Corps of Engineers (USACE), who had been geoengineering erosion control blankets for marine projects since the 1980s (Allen & Leech, 1997). The U.S. Forest Service (USFS) applied similar techniques in the late 1970s in post-retention harvest units for streambank erosion control (Allen & Leech, 1997).

Perhaps the only terrestrial wetland restoration-specific study that applied pre-vegetated mats with coconut-coir since the turn of the 21<sup>st</sup> century was an experiment that used coirmatting to improve embankment vegetation quality, marsh terracing (bank stabilization), and erosion control in a wetland heavily invaded by PHAR (Hook et al., 2009). In this seminal study, coconut-coir mats were applied in a variety of forms (bio-tubes, terraces, pre-vegetated mats) to restore a wetland habitat to its natural state after multiple years of PHAR invasion. The bio-tubes were installed along bank sides to provide erosion control and native species revegetation. The bio-tube swales extended the bank inwards, narrowing the channel width, and increasing terrestrial space. This ultimately resulted in PHAR reinvading the coir-terraced terrain after a single growing season by a greater terrestrial extent than it had before restoration took place (Hook et al., 2009). However, first year results along some of the banks of the study area exhibited rapid growth of herbaceous (primarily sedges) and woody species within coir-mats and erosion control coverings, indicating that the mats potentially boosted native vegetation establishment over that of PHAR (Hook et al., 2009).

# Conclusion

Consensus on best practices for PHAR control has yet to be reached in the scientific community. Appropriate treatments are highly site-specific, as they must account for site hydrology, native flora and fauna present and other historic and current disturbances. Regardless of the frequency of studies that repeat longstanding maxims of a handful of authors, there is always more to be gained by a thorough investigation of the less-investigated options. For chemical and mechanical techniques especially, a general lack of consistency among study designs, geographical location, duration, and replication is well noted (Kettenring & Adams, 2011). Discovering new techniques in a locally relevant context can establish an acumen of

confidence for restoration practitioners seeking new ways to apply techniques, opening avenues through which the subject can grow, a knowledge gap identified shortly after the turn of the 21<sup>st</sup> century (Perry & Galatowitsch, 2003). However, some agreement exists in the literature on several key points pertaining to PHAR control in ephemeral wetland habitats, including remnant/historical hydrological regimes necessary to support native revegetation over the longterm following the restoration phase.

Interconnected wetlands with a history of disturbance are frequently re-infested by PHAR on an annual basis from the influx of proximate seed sources during seasonal flooding inundation and other disturbances. The PHAR reinfestations often co-occur with natural or modified hydroperiods and flooding such as storm surges when the ordinary mean high water level is breached, sometimes occurring multiple times per annual cycle (McAllister & Leonard, 1997; Richardson et al., 2007). These problems factor into the difficulty of controlling PHAR, which, having attained circumboreal invasive status since the industrialization of the modern world on account of its superior genetic plasticity, including those relating to its adaptation in favor of our warming climate, has continued to resist abatement under any anthropological pressures attempted to date.

## **CHAPTER 2: RESEARCH MANUSCRIPT**

Degradation from the spread of invasive species in lowland, palustrine-emergent riparian habitats is one of many factors that contributes to the loss of biodiversity in North American wetlands (Bennett et al., 2011). Land use, development, and other human disturbances are responsible for wetland losses in the United States (U.S.), which, in turn, have disproportionately affected some specialist species of herpetofauna (Halliday, 2013; Stuart et al., 2003). Global biodiversity has declined by over twenty percent (> 20%) of its pre-industrial state since the turn of the 20<sup>th</sup> century, with the greatest decline seen among amphibian species (> 40%) (Brown et al., 2012; Diaz et al., 2019; Stuart et al., 2003). Rana pretiosa (Oregon spotted frog: OSF) has been highly impacted by these changes, as evidenced by the steep decline of its historical range by over seventy percent (>70%) since the early 1980s (Hayes, 1997). Some wetland habitat features that the OSF requires include the presence of shallow pools, breaks and gaps in canopy cover, and low-lying emergent vegetation to support reproduction, metamorphosis, and hibernation activities throughout its life cycle (Kapust et al., 2012; McAllister & Leonard, 1997; Padgett-Flohr & Hayes, 2011; Watson et al., 2000). The total area of intact wetlands capable of supporting the OSF under these criteria has been considerably reduced in the Pacific Northwest, making restoration efforts critical to its recovery (Halliday, 2013; Hallock, 2013; Horvath et al., 2017).

Of particular concern to OSF restoration efforts are the ecological impacts from accelerated wetland loss in the northwestern U.S., where, among other factors, invasive *Phalaris arundinacea* (Reed canary grass : PHAR) has monopolized most of the remaining critical habitat suitable for this species, primarily encompassing lowland open-canopy and ephemeral wetlands (Hook et al., 2002; Kapust et al., 2012). Since many amphibians have specialized habitat

requirements, including precise hydroperiods and thermal thresholds for breeding and larval survival, restoration designs that consider the specific habitat requirements of declining wetland species are more likely to establish long-term anuran persistence (Brown et al., 2012). Restoration practices have the potential to offset some of these losses, though existing literature indicates a need for continuing research into integrative methods, beyond traditional methods of herbicide and mowing, to counteract PHARs invasive mechanisms and establish native vegetation that can effectively compete in the face of persistent PHAR assault.

An alternative restoration option was revisited in this study to evaluate the effects of biodegradable coconut coir mats on native wetland species establishment and PHAR. The emergent vegetative mat (EVM) (frequently termed 'coir-mat' or 'coconut-coir mat' in the literature), has existed for several decades, with use possibly predating the 1980s, when coir mats were used in U.S. Army Corps of Engineers (USACE) and U.S. Forest Service (USFS) projects for streambank and marine environment erosion control (Allen & Leech, 1997). However, reference material on the EVM is practically non-existent in ecological restoration literature. Information is particularly scarce for northwestern U.S. applications of vegetated mats in varying wetland hydrology, for conservation, and in support of habitat conditions that correspond to specialist amphibian breeding habits and survival. Since the OSF is a specialist amphibian species that seems rather sensitive to changes in its preferred habitat, most of which has either disappeared or become homogenized over time by invasive species, examining the EVMs in emergent-palustrine and ephemeral wetlands for restoration purposes has broadscale relevance for wetland ecology and wildlife conservation. Therefore, it follows that further documentation on EVM applications in Pacific Northwest wetlands would fill a significant gap in the literature.

I hypothesized that EVMs have the potential to preserve habitat characteristics of the imperiled OSF, while simultaneously enhancing wetland functions through the suppression of PHAR's early competitive growth under the following assumptions: 1) Native species plantings in the EVMs can survive through PHAR's competitive early season growth, meeting OSF habitat criteria for heterogeneous vegetation composition provided by low-stature vegetation; 2) The pre-planted EVMs successfully adhere to the soil substrate for at least three years under varying hydrology and in the advent of storm events and floods; and 3) EVMs will provide sufficient PHAR suppression for low maintenance, enabling sensitive amphibian species to breed at the restoration site with little disturbance.

To better understand the effectiveness of EVMs as part of an integrated wetland restoration strategy for OSF habitat, I sought to answer four questions:

- 1) How effective are EVMs at establishing native vegetation?
- 2) How effective are EVMs at suppressing PHAR cover?
- 3) Which restoration treatment methods were most effective at limiting PHAR prior to EVM placement?
- 4) Which hydrology is associated with the best outcomes?

#### **Materials and Methods**

# Study Sites

This study was conducted at three PHAR-invaded seasonal wetlands sites in the lower Puget Sound region of Thurston County near Olympia, WA. These include: 1) Mima Creek Preserve (MC), which is owned and managed by the Center for Natural Lands Management; 2) Watkins Marsh (WM), which is owned and managed by Joint Base Lewis-McChord (JBLM); and 3) West Rocky Prairie (WR), which is owned and managed by the Washington State Department of Fish and Wildlife (WDFW) (**Figure 2**). The sites were selected for their suitability at supporting OSF, with particular emphasis on the habitat features and site conditions needed for oviposition and metamorphosis. Both MC and WR are currently OSF-occupied. For the hydrological component of this study, experiment plot areas at each site were selected for their relative hydrological uniformity at three inundation-depth levels (in terms of mean average water level per year by hydrology zone as opposed to elevation or slope), as well as the likelihood that they would support the native target species selections in the mats.

Figure 2 EVM Study Site Locations



*Note.* Map of study site areas from left to right: Mima Creek Preserve (MC), West Rocky Prairie (WR), and Watkins Marsh (WM). ArcGIS Online Living Atlas National Geographic base map in datum: WGS 84 Web Mercator Auxiliary Sphere. Projected coordinate system: WGS 1984 UTM Zone 10N. (ESRI ArcGIS Pro, v2.1.0)

## **EVM Production**

The EVMs were pre-planted with native Washington State wetland plant species in a greenhouse setting prior to installation by corrections inmates and staff at Stafford Creek Corrections Center (SCCC) as part of the Sustainability in Prisons Project (SPP). The coconut-coir EVMs (Bio-D Pillows, Rolanka International) were customized in dimensions of 9-m<sup>2</sup>. Two species of emergent macrophytes and one wetland obligate grass were initially planted as rhizomatous propagules in the EVMs, kept saturated on large hydroponics platforms and grown for six months before transport and installation.

The species selected for this study included *Eleocharis palustris* (Common spike-rush: ELPA), *Juncus supiniformis* (Spreading rush: JUSU), and *Glyceria elata* (Fowl mannagrass: GLEL). Each species was chosen for its potential to compete with PHAR in degraded wetland systems. Specifically, GLEL was chosen for its competitive vegetative height, ELPA for its potential to form dense rhizomatous growth proportional to its aboveground biomass, and JUSU for its widespread prevalence in the region and respective hardiness under competitor pressure and deep hydrology.

### **EVM Placement**

In Fall 2017, the EVMs were transported from SCCC to the study sites as rolls and installed. Three replicate EVM and control arrays per water depth zone were installed at each site, constituting a total of 9 EVMs per site and 27 mats across all sites (**Figure 3**). Corresponding control plots that received vegetation management treatments but no EVMs were placed 2 m away from each EVM and delineated by pin flags. This enabled us to evaluate the effectiveness of the EVM restoration treatment combinations against standalone restoration treatments.

# **Restoration Treatments**

Separate restoration treatments were assigned to each study site as follows: MC, solarization and mowing; WM, herbicide and mowing; WR, mowing. Since MC and WR were known to have recent OSF breeding activity on account of egg masses found at both sites prior to 2017 EVM installation, these sites received non-chemical restoration treatments. Treatment area in hectares (ha) for each study site were as follows: MC = 1.2 ha, WM = 0.8 ha, and WR = 0.6 ha (**Figure 3**).

# Figure 3 EVM Placement by Site



*Note.* 2017 EVM installation and pre-treatment areas at each site. From left to right: Mima Creek (MC), Watkins Marsh (WM), and West Rocky Prairie (WR) on day of EVM installation (Hamman, 2018).

# Mima Creek

The pre-EVM installation restoration prescription for MC involved an installation of white solarization plastic that was pinned over the full treatment area at each hydrology depth using metal stakes. The solarization treatment was only applied during the summer of 2017 after the initial pre-treatment mowing application that occurred in July 2017. Solarization plastic was in place from 29 July through 30, September 2017 (2 months). The EVMs were installed on 17 October 2017 over the mowed and solarized pre-treatment areas.

Similar to the mowing applications for other sites, MC mowing was applied with smallengine brush-cutters that had steel tri-blade and circular blade attachments to mulch PHAR aboveground growth, first by mowing stems close to the ground, and then cutting the thatch layer into chunks. This enabled the thatch to be raked away from treatment plots so direct contact between bare soil and the pre-planted plugs could be achieved for solarization plastic treatments and mat installations. Mowing was applied again to the full treatment area on 17 October 2017, the day of EVM installation at MC. Thereafter, annual mowing treatments were applied in the summer between July and early October of 2018 and 2019 to the areas within and around the EVMs. For all follow up treatments post-EVM installation, mulching material was left on site, including within EVM treatment plots.

#### Watkins Marsh

WM was also brush-cut prior to both herbicide application and EVM placement in 2017, as PHAR vegetative height would have reduced the effectiveness of the chemical treatment. Handheld backpack applicators were used to apply the prescribed herbicide formulation of (1%) imazapyr (Aqua-Master®) and one percent (1%) oil-based surfactant (Agri-Dex®) per three gallons (3 gal) of water (H<sub>2</sub>O). First year (2017) herbicide treatments at WM were applied twice, with initial herbicide treatments applied in the spring, and the latter occurring on 19 October 2017, just before EVM installation. Thereafter (2018 – 2019), all herbicide treatments were applied around two weeks after brush cutting treatments had occurred and PHAR regrowth had attained greater height than the mowed thatch. Additionally, WM mowing and herbicide treatments occurred only around the mats (not within), apart from 2017 mowing in preparation for EVM installations.

## West Rocky Prairie

The vegetation management treatments at WR involved mowing PHAR once annually in the early fall. Mowing was initially applied to the full treatment area at WR in autumn, 2017 before EVM installation. Similar to the vegetation management plan for MC, WR EVM plots were mowed in addition to the surrounding treatment area.

## Monitoring

To evaluate the performance of EVMs in PHAR infested wetlands for long-term for OSF habitat management objectives, the effects of different restoration treatments on native vegetation and PHAR cover at different hydrology depths were monitored annually from 2017 to 2019 in the late spring to early summer (May – July). The final vegetation monitoring period occurred between 16 June and 6 July 2019 at all three study sites.

Vegetation monitoring methods for 2019 were derived from the sampling design used for previous years (2017 - 2018) as well as principal reference literature (Elzinga et al., 1998). Proportional cover estimates of native target species and PHAR were taken by sampling vegetation cover in the subplots of each EVM array (4 subplots / EVM plot) per water depth, including control plots (Appendix C.

**Table C 1**). Cover classes were visually estimated according to designated intervals for native species GLEL, JUSU, and ELPA, with PHAR cover monitored separately. Minor differences existed between the monitoring protocol for 2017 and 2018 compared to 2019 (**Table 1**).

2017 - 2018 Protocol				2019 Monitoring Protocol						
Native S	Species	& PHA	R Cover	Native Species Cover			PHAR Cover			
Bin (%)		Value	to Record	Bin (%)		Value to Record	Bin (%)		Value	to Record
0-1		0.5		0-1		0.5	0-1		0.5	
1-5	••••	3		1-5		3	1-5		3	
6-10		8		6-10		8	6-10		8	
11-20		15		11-50		30	11-20		15	
21-30		25	(3595)	51-99		75	21-30		25	(3595)
100		100		100		100	100		100	

 Table 1 Monitoring Protocol for 2017–2018 and 2019

*Note*. Monitoring protocol for study time series for 2017 - 2018 (left heading), and 2019 (right heading) showing binned intervals for % cover estimates of *Phalaris arundinacea* (Reed canary grass: PHAR) and pre-planted native species in EVMs. 2019 native species cover classes were changed from original protocol in the 4th (11-50) and 5th bins (51-99).

The field protocol for vegetation data collection involved placing a  $1-m^2$  polyvinylchloride (PVC) quadrat frame over the subplots in each array to aid with visual estimations of proportional cover classes for each target species in intervals between zero and one hundred percent (0 - 100%) (**Figure 4**).

Additionally, extraneous (non-target) vegetation cover estimates inside the EVM treatment sub-plots were collected during the monitoring period of 2019. Samples of unidentified taxa in the EVM treatment plots were taken during 2019 monitoring and identified using standard taxonomic keys from Hitchcock & Cronquist (1973) (*see* Appendix A, **Table A 1**).



Figure 4 Emergent Vegetation Mat (EVM) Schematic Sampling Design

*Note*. Emergent Vegetation Mat (EVM) Schematic Sampling Design. Experimental monitoring design of one replicate set of EVM (left) and its corresponding control plot (right), showing four 1-m<sup>2</sup> monitoring plots (dark crosshatch) within 9-m<sup>2</sup> EVM mats (black dots), enclosed by a 16-m<sup>2</sup> buffer strip of Reed canary grass (PHAR) treatment area (blank). Adapted from Hamman (2018).

Water depth (cm) and air temperature (C° F) data were collected during the OSF oviposition period (February – March) each winter from 2017 - 2019 with HOBO U20L data loggers (Figure 5). Hydrology intervals were set at shallow, moderate, and deep-water levels corresponding to the OSFs oviposition habits and measured as follows: 5 - 12 cm (shallow), 13 - 25 cm (moderate), and 26 - 40 cm (deep) according to 2017 to 2018 water depth data collection.

Three rigid polyvinyl-chloride (RPVC) monitoring wells installed at each of the three water depths per site were used to house and protect the dataloggers. The data loggers were installed by attaching aluminum weave cording to data loggers and lowering them through the mouth of the well to intercept the depth baseline of the water table. As water levels changed, the data loggers registered the change in pressure and temperature at one-hour intervals. One air
pressure data logger was suspended at the top of one well at each site to provide a standardized metric for conversions to water depth after the data loggers were collected.





*Note.* 2017 Hydrology Depths (m) and Temperature (°C) by Site: Left y-axis shows temperature (°C) in intervals of ten (-10 - 40 °C), right y-axis shows hydrology depth (m), and x-axis shows month (October – June) in each hydrology zone (Deep, Moderate, Shallow) at each site.

Additionally, surface temperatures were taken at MC during the summer of 2017. Two HOBO U20L dataloggers were installed in each of three EVM arrays per water depth. One was attached outside the solarization plastic to monitor surface temperature (°C) and the other to collect ambient heat underneath the plastic (°C). The temperature frequency of the solarization dataloggers was set to collect every two hours. Temperatures under the plastic (avg. 18 °C, max 60 °C) were consistently higher than those adjacent to the plastic (avg. 17 °C, max 53 °C) (S. Hamman, personal communication, 4 January 2018).

#### Data Analysis

Data processing, organization and analysis was performed in Version 3.6.1 of the opensource statistical graphing software program R (R Core Team, 2019). Hard copy datasheets were used in the field to document cover estimates of each species in the study: GLEL, JUSU, ELPA and PHAR, and then manually transferred into Microsoft Office Excel and R. Site and species range maps were created in the ERSI<sup>®</sup> mapping software program ArcGIS Pro, version 2.8.0. Graphs were digitally reformatted and modified for accessibility purposes with vector software programs GIMP<sup>®</sup>, VECTR<sup>®</sup>, and Inkscape<sup>®</sup>. Initial summary statistics of 2017 to 2019 monitoring data were computed using R packages: ggplot, dplyr, tidyr, and purr.

The explanatory variables in this study are restoration treatments (solarization, herbicide, mowing), hydrology depths (shallow, moderate, deep), the EVM treatments, and time (2017 - 2019). The response variables were cover estimates of target native species plantings and PHAR in the EVM and control plots. The EVM treatment sample sizes were equal for all response groups (n = 108) in annually consistent population sizes (N = 324) per response variable over all three monitoring years. The presence of multiple explanatory groups and response variables led to processing the initial statistic with a 2-Way Analysis of Variance (2-ANOVA) model, where paired control and treatment plots were nested in arrays corresponding to each hydrological depth per site, with repeated measures by year.

From the initial statistical output, it was determined that the data did not fit the assumptions of a 2-ANOVA model by: a) having a normal distribution, or b) equal variances throughout. In addition, the data were zero-inflated, as is sometimes typical of visual vegetation

cover estimates organized by proportional cover classes. An arcsine transformation was applied to normalize the dataset, followed by a Kruskal-Wallis rank sum test with a 95% confidence interval with two degrees of freedom (DF = 2,  $*p \le 0.05_{0.95}$ ) when it became evident that the applied log transformation had not resolved heteroskedasticity. The Kruskal-Wallis *p*-values were adjusted with the Holm method for multiple comparisons, showing the previous model did not fit the data and further testing was required.

For the final analysis, we used a Bayesian hierarchical beta-regression model to 1) estimate % cover of PHAR in each site  $\times$  depth (hydrology)  $\times$  year combination with 95% highest posterior density (HPD) intervals, and 2) test whether that % cover of PHAR was modified by the vegetation mat treatment (relative to control plots) in each site  $\times$  depth  $\times$  year combination. Binned PHAR cover (converting 0-100% to 0-1 values) was used as the response variable with a logit link post-hoc transformation. The logistic transformation accounted for zero-inflation by fitting the continuous PHAR data with the Markov chain Monte Carlo (MCMC) method on a negative binomial scale (see Appendix C, Equation 1) (Hadfield, 2010; Irvine & Rodhouse, 2014; Plummer, 2019). Bin-widths that were part of the original design needed to be adjusted to reduce zero-inflation of the dataset. Since PHAR data were the most zero-inflated, the bin intervals were changed to 0, 0.01, 0.05, 0.10, 0.20... 0.90, 1.0 (see Appendix C, **Table C 1**). This resulted in a 'smoothed' PHAR response variable, wherein binned data were accounted for by generating a continuous value at every iteration. With this method, the combined midpoints of sample population data for each EVM per hydrological depth array served as the statistical indicator of their effectiveness at suppressing PHAR in varying inundation levels. The calculation and visual output of the beta regression were generated using R packages rJAGS (v4.1.0), glm, MCMC, betareg, lattice, and lecuyer (Kellner, 2017).

## Results

### Native Species Response by Site, Depth, and Year

Native species cover in the EVMs over the three years of this study was variable, but a few important differences merit comment (*see* Appendix C, **Table C 2**) (**Figure 6**). With two exceptions, cover of *Glyceria elata* (GLEL) and *Eleocharis palustris* (ELPA) was generally low. The exceptions were: 1) median GLEL cover was highest in the deep hydrology treatment at MC during 2019 (median ~ 36%), and 2) median ELPA cover was elevated in the moderate depth hydrology treatment at WR (median ~ 15%). In contrast, *Juncus supiniformis* (JUSU) had greater cover on average than either GLEL and ELPA across a majority of hydrology treatments and years. However, all three species had low cover at shallow depths at the mow only site (WR, all years), and at the shallow depth hydrology treatment at MC in 2019. At WM, some cover of JUSU persisted across all hydrology depth treatments and years, with medians of at least 20% by 2019. By 2019, JUSU cover was only present in all three hydrology depth treatments at WM, the site that had received herbicide treatment (**Figure 6**).

### Reed canary grass (PHAR) Response

Estimates of % PHAR cover with 95% HPD intervals reveal a re-establishment of high cover levels (median >55% by 2019) at MC (solarization – mowing treatment) and WR (mowing only treatment) across all depths in both treatments and controls (*see* Appendix C, **Table C 2**;

Figure 7). In contrast, WM (herbicide treatment) had relatively lower % PHAR cover (median  $\leq 26\%$ ) (*see* Appendix C, **Table C 2**; Figure 7) across all depths and years; notably, PHAR response in the coir mat treatments were somewhat greater than the controls. Interestingly in 2019, the shallow depth hydrology treatment at West Rocky had greater % PHAR cover in the

coir mat treatments than in the controls (non-overlapping 95% HPD intervals) (*see* Appendix C, **Table C 2**).



Figure 6 EVM Treatment Effects on Native Species Cover (%)

*Note*. Native Species % Cover in Vegetation Mats (right y-axis) by Site (left y-axis), Water Depths (top), and Year (on x-axis). Key: GLEL (blue) = *Glyceria elata*; JUSU (pink) = *Juncus supiniformis*; and ELPA (green) = *Eleocharis palustris*. Midlines in boxes = sample medians (x); Boxes = Data quartiles  $Q_1$ - $Q_3$ ; Whiskers (dotted lines) represent the subsample range (0-100); minimum - maximum.



Figure 7 EVM Treatment Effects on PHAR Cover %

*Note.* Reed canary grass (*Phalaris arundinacea* /PHAR) % Cover (right y-axis) in Control (C: blue) and Emergent Vegetative Mat (VM: pink) Plots by Site (left y-axis), Water Depth (top), and Year (on x-axis). Midlines in boxes = sample medians (x); Boxes = Data quartiles  $Q_1-Q_3$ ; Whiskers (dotted lines) represent the subsample range (0-100); minimum - maximum.

For the analysis estimating PHAR cover in the EVMs relative to control plots, both the raw data (**Figure 7**) and treatment effects from the beta regression (**Figure 8**) are informative. Except for the shallow depth hydrology treatment in 2019, solarization prior to mat installation at

MC was the only treatment that resulted in any significant decrease in PHAR cover (relative to controls; **Figure 7**, **Figure 8**). This effect was consistent across the three hydrology depth treatments (**Figure 8**). That said, even at MC under the EVM treatment, PHAR cover by 2019 was relatively high (median cover >50%, **Figure 7**). This contrasted with PHAR cover at WM, where median PHAR cover was < 30% for both control and EVM treatments (**Figure 7**). The data reveal that hydrology (inundation level) lacked a clear impact on PHAR or native species cover. (**Figure 7**).



Figure 8 Beta Regression Results for EVM Treatment Effects on PHAR

*Note*. Beta-regression plot output showing the combined treatment effects and EVM response on PHAR by site (top), depth (x-axis), and year (for each depth, results are plotted in the order 2017, 2018, 2019). The y-axis represents a logistic scale, where the midline (0), is "not significant," and the beta-coefficient midpoints (blue diamond:  $\mu$  = predictor variable), fall between about -10 and 10 (below midline = reduced PHAR cover in EVMs relative to controls; above midline = increased PHAR cover in EVMs relative to controls).

### Non-target Native and Exotic Species Richness

Non-target native species richness data were collected for 2019 monitoring only. In addition to PHAR and the three native species in the EVMs, a total of 11 other native species and 14 non-native species were detected across all sites, depths and treatments (see Appendix A, **Table A 1**). Out of all study sites there was a greater variety of non-target taxa observed at WM, but most of these were non-native. Non-target exotic species monitored in 2019 at WM included: Cirsium arvense (Canada thistle), C. vulgare (Bull thistle), Ranunculus repens (Creeping buttercup), and *Trifolium dubium* (Lesser trefoil), among others (see Appendix A, Table A 1). Non-target native species richness at WM was highest, with several obligate and facultativewetland forbs observed, including Veronica americana (American speedwell), and Myosotis micrantha (Small-flowered forget-me-not), M. scorpioides (True forget-me-not), and Pseudognaphalium stramineum (Cotton-battling plant). However, abundance of these herbaceous species was generally low. Additional non-target native species at WM included seedling cover of several woody species (not included in non-target data collection per protocol, but prolific enough to take note of), including: Alnus rubra (Red alder) and Pseudotsuga menziesii (Douglas fir). The highest non-target native species abundance was observed at WR, with *Carex obnupta* (Slough sedge) dominating inside the EVMs and controls, and around plots in the mowed treatment areas, and Juncus effusus (Common rush) present at all inundation depths. C. obnupta cover estimates were as high as 75% in some of the shallow and moderate monitoring subplots. Comparatively, non-target species richness was much lower at MC than the other two sites in both treatment and control plots at all inundation depths.

## **CHAPTER 3: DISCUSSION & CONCLUSION**

# Discussion

This study summarizes one of the first Pacific Northwest-specific evaluation of the emergent vegetative mat (EVM) for ecological restoration purposes in recent years. As discussed in the literature review, the extent to which EVMs have been thoroughly evaluated for their use in ecological restoration is minimally evaluated at best. EVMs may be useful in specific circumstances, but future studies should evaluate how more intensive pre- and post-EVM installation treatments perform over a longer time interval. Future trials under these conditions would obviously require project managers to guarantee more funding, but it has become increasingly difficult to allocate project money with the ever-decreasing state of federal funding sources.

The results from this study confirmed previous assumptions in the literature about the likelihood of Reed canary grass (*Phalaris arundinacea*: PHAR) reinvading following disturbance from restoration activities, reinforcing the evidence for the necessity of intensive retreatments for multiple years post-restoration (Anderson, 2019; Kaproth et al., 2013; Matthews et al., 2020; Tu & Salzer, 2004). For one, the EVM did not seem to give native plantings a particular advantage over PHAR in heavily invaded, seasonal wetlands without a more intensive maintenance plan to control annual PHAR resprouts. This outcome is also characteristic for many traditional methods used on PHAR in heavily invaded habitat. Since PHAR is such a formidable invasive, continuing to pose challenges to land management over decades with little improvement in the progression of methods for its control, additional research on restoration methods that are both integrative and aggressive is needed.

Secondly, no clear trend in the results indicate which of the three water depths used in this study aided in the establishment of native species. This is a component of the study that merits further investigation for land managers who are still interested in employing the EVM technique. Some moderate and deeper hydrology plots in two of the study areas had a mixture of greater native species cover and/or non-target native species diversity compared to EVMs installed in shallow plots. Because PHAR can spread even at the deepest inundation levels we addressed (deep hydrology), it is possible that, following a more intensive PHAR control regiment, taller emergents or obligate grasses like *Glyceria elata* (GLEL) could establish more readily in deep hydrology than the other species of shorter stature used in this study (Coops et al., 1996).

Mowing treatments applied in West Rocky (WR: mowing only) treatment plots were relatively ineffective on overall PHAR cover by the third year, but either due to pre-existing site characteristics or some environmental changes (i.e., open canopy allowing light to stimulate germination of the preexisting native seed bank) native species richness was higher here than at Mima Creek (MC: solarization and mowing). Imazapyr applications applied post-mowing at Watkins Marsh (WM: herbicide and mowing) showed some success in reducing PHAR cover for long enough to establish *Juncus supiniformis* (JUSU) cover in shallow and moderate inundation depths throughout all three years of the study series at all sites. However, high post-application mortality of native species plantings had occurred at WM by 2019, and a significant portion of GLEL and JUSU showed signs of epinasty from the herbicide treatments. Consequently, some indication exists that JUSU had initial survival at all three sites, depths, and treatments relative to the other target native species plantings in this study but did not retain its cover at all water depths at WR or MC over the treatment series. It was difficult to tell if solarization had a significant effect on MC monitoring results by year two or three, as the treatment was not applied again after mat installation occurred in 2017. However, the location of MC on an open flood meadow along the Black River could have exposed the EVMs to greater hydrological inundation levels as well as a continual influx of PHAR seed during those periods, so it cannot be assumed solarization would not have been appropriate for the other sites on account of their different characteristics. The mowing treatments at WR were unremarkable both in terms of native vegetation cover gains and PHAR cover reductions. Thus, the only treatment prescription that showed signs of benefiting native species establishment in the EVMs was the herbicide and mowing regime at WM.

Additionally, the results from 2019 monitoring implied a general decline of gains acquired in JUSU cover over the previous years of treatments (2017 - 2018). The concentration of imazapyr to H<sub>2</sub>O (> 0.5% tank mix) may have played a role in the residual long-term effects of this selective herbicide on native species plantings. To illustrate this point, less than one percent imazapyr and four percent glyphosate active ingredient per three gallons of water is potent enough to eradicate large monocultures of the highly invasive broadleaf Knotweed species (*Fallopia* spp. [var] *Polygonum*).

Perhaps the most important consideration of this research involves imparting a general understanding to land managers, restoration ecologists, and other practitioners about some of the details about the EVM that can influence a thorough cost-benefit-analysis. While coir geotextiles have been well documented for civic engineering purposes, more research is needed on the use of EVMs in purely restoration-based situations. Multi-year experiments beyond the scope of this study could enhance knowledge of the EVM's long-term efficacy, excluding the installation year (in this case, 2017) from the total time series to eliminate biases that inevitably arise from

including first-year monitoring data. If the installation year is excluded from monitoring and results, a four-year study on the EVM could capture vegetation responses to treatment combinations throughout the anticipated degradation timeline of coconut coir matting products: estimated at 1-3 years (Banerjee, 2020). The time series extent of this study was relatively short due to funding constraints, but it provides a basis of understanding on the site preparation and treatment results for start-up restorations.

A cost-benefit analysis of international suppliers and other shipping companies that provide coir products at a lower cost or higher quality of fiber could incentivize land managers and other practitioners to experiment with EVMs regardless of the results of this study, as the cost per square meter) of processed coir ( $100 - 133/m^2$ ) from our supplier at Sustainability in Prisons Project (SPP), is currently too expensive for most restoration projects en masse. Additionally, it could benefit current suppliers in the Pacific Northwest to have a cost analysis done for them by another entity. For example, a future student could tackle this project, permitted the individual is allowed to access their facility and communicate with staff and inmates at Stafford Creek Corrections Center (SCCC) to discuss ideas workers may have about cutting production costs. The obvious cost-benefit SPP has from contracting inmate labor to produce pre-planted EVMs could provide a window of opportunity for assessing different merchandizing strategies in the future. To highlight an aforementioned example, the permeable nature of coir geotextile permits light to facilitate one of PHARs most potent invasive mechanisms: axial tillering early in the growing season in advance of native species emergence (Hayes et al., 2013; Iannone et al., 2008b; Reinhardt Adams & Galatowitsch, 2005). Thus, it could benefit managers interested in using the EVM technique to explore different design options in the market that could better suppress PHARs rhizomatous emergence, especially since

PHAR has been shown to be sensitive to light deprivation in greenhouse experiments (Kneller et al., 2007).

Some consensus exists in the literature that problems associated with common PHAR control methods could be ameliorated through the investigation of integrative restoration techniques that have received less attention in the literature (Capon & Pettit, 2018; Corbin & D'Antonio, 2012; Lowry et al., 2013). Many publications dealing with the underlying biological mechanisms that fuel PHAR's invasiveness have argued for improved cross-institutional communication, inter-agency collaboration, and data sharing that could advance the proliferation of ecosystem-specific, locally relevant information science repositories (Adams & Galatowitsch, 2006; Dudgeon et al., 2006; Horvath et al., 2017; Lavergne & Molofsky, 2006; Taddeo & Dronova, 2018; Thorslund et al., 2017).

Regarding the specific application of EVMs for OSF habitat restoration in PHAR dominated wetlands, no compelling evidence exists from this study to advocate deploying this technique for such purposes, as it may be counterproductive and is not recommended at this time. The vegetation cover results for WM herbicide and mowing treatments exhibited the highest level of PHAR reduction results by the second and third treatment years compared to the other study sites/treatment combinations. Regardless of these results, it would be premature to unambiguously conclude that the EVM - imazapyr treatment combination should be recommended over other available options for either OSF habitat restoration and/or PHAR control. First, herbicide applications with aquatic formulations of imazapyr are still not permitted in any habitat known to currently harbor OSF populations on a federal level. Imazapyr treatments were permitted at WM because OSF has never been documented as present at this site. It follows that imazapyr must first be approved for use in OSF habitat before recommending

this treatment regime, and that alternate herbicide selections would have to include both those that are systemic in their mode of action to translocate into rhizomes, and environmentally persistent enough to control PHARs persistent regrowth between treatments. The PHAR annual growing cycle starts as early as March and can extend through late October (or whenever the first frost occurs) in parts of the Pacific Northwest. As examined in the literature review, few herbicide options exist to date that perform quite as well as imazapyr that are approved for aquatic application in current OSF habitat and have no demonstrated lethal or sublethal toxicity to amphibians. Washington State Departments of Ecology and Fish and Wildlife do not currently provide clearance for imazapyr application in OSF-inhabited areas, yet aquatically approved glyphosate formulations are permitted in Washington State despite its documented toxicity to anurans (Healy & Zedler, 2010). Therefore, resolving any research gaps that are preventing the use of imidazoline herbicides in OSF habitat could potentially assist in this endeavor (M. P. Hayes, personal communication, 30 July 2020).

Some functional concerns also exist with EVMs that should be investigated further before recommending this method for use in PHAR dominated wetlands, including reservations concerning its application in designated critical OSF habitat. For one, some concern exists that the mats may facilitate PHAR invasion rather than suppress it. As explained in the literature review, this concern was expressed by one of the EVMs earliest proponents (Hook et al., 2009). Hook et al., (2009) cautioned against the application of coir-pith bioswales (bio-logs) in direct line of hydrological flow after first year monitoring revealed that the bio-logs had increased PHAR's spatial extent on site. The bio-logs, which had been installed along the margins of a stream and planted with native vegetation, caught the first influx of PHAR seeds from upstream flows when seasonal water levels rose. By the following spring PHAR had invaded all planted

vegetation areas as well as the installed erosion swales, collecting enough sedimentation from the influx of winter storm surges to physically narrow the stream channel (Hook et al., 2009). This draws attention the issue of PHAR's strong invasive mechanism via hydrology, whereby seed deposition occurs in the event of storm surges, continually manifesting in overlapping, annual infestations (A. Martens, personal communication, 15 June 2019). Some EVMs at WR were so buried by sedimentation from seasonal inundation periods and PHAR cover that it was nearly impossible to find the mats without assistance from those involved in their installation. Handremoving thatch before spring emergence of native vegetation could aid restoration efforts in later spring and early summer monitoring and retreatment activities in heavy PHAR cover, and potentially reduce some of the shade suppression effects of PHAR thatch on native vegetation establishment. Specifically, this can be exemplified by the results of the shallow plots at MC which seemed to respond negatively to the EVMs in terms of the ratio of native vegetation cover to PHAR cover. This observation was also observed in WR native vegetation monitoring data by 2019, which had heavy sedimentation load with little native vegetation cover proportional to PHAR extent of reinvasion in the mats. Even MC plots in deep hydrology had evidence of new PHAR growth from seed. Low thatch accumulation and a high volume of seedlings deposited on the surface of the EVMs at all depths (deep plots especially) at WM indicated that the site potentially experiences fast flowing hydrology and prolonged inundation on account of the deep hydrology arrays, which stood like an island of pure PHAR within a vegetation-bare imazapyr treatment area.

## Conclusion

This study was one of the first Pacific Northwest-specific evaluations of EVMs for ecological restoration purposes. This trial fulfills a preliminary gap in the literature on a

technique that has been poorly evaluated. As discussed in the literature review, the extent to which the EVM has been thoroughly evaluated as an ecological restoration tool is minimal at best. EVMs may prove to be an ideal restoration tool under specific circumstances, but further research is needed to establish their niche in ecological applications. Future studies should evaluate how more intensive PHAR treatment plans perform on vegetation cover over a longer time series experiment.

What is clear from the results is that pre-planted EVMs do not provide enough PHAR suppression to establish two of the chosen native species in the mats over a period of three years (aside from JUSU, which showed some potential at WM with imazapyr treatments) without a much more intensive post-installation re-treatment schedule over multiple years. For example, the herbicide and mowing treatment regime at WM appeared to favor incrementally 'higher' JUSU cover in the EVMs over time, but the proportional nature of the monitoring design did not account for the presence of thatch or bare-ground space in the plots for computational reasons (pseudo-binary data). The inclusion of these other variables observed during monitoring periods in a separate test could possibly provide a more accurate vegetation cover estimation in response to the other explanatory variables, such as hydrology. Additional assumptions about the results at WM could have arisen from its contrast to MC and WR (non-herbicide treatment sites), both of which had almost no bare ground space, but had accumulated high thatch content. As noted earlier in the literature review, thatch accumulation serves as one of PHARs most effective invasive mechanisms (Foster & Wetzel, 2005; Kercher & Zedler, 2004), Thus, it is possible that while the results of PHAR cover across all sites and treatments were variable, the effective thatch removal via herbicide facilitated JUSUs continued survival into 2019.

A more thorough treatment regime will require a larger budget: a prerequisite considered in most restoration project proposals that is highly dependent on federal funding and other forms of inter-organizational support (Hierro et al., 2005). Additionally, owing to PHARs propensity to readily adapt in response to varying environmental changes, its prolific seed bank and production, and aggressive rhizomatous spread, management of this grass should consider multiple options and incorporate integrative methods for its control. Therefore, it remains crucial for practitioners to narrow down which restoration techniques are the most effective at suppressing PHAR cover in OSF habitat. Future studies on effective restoration techniques for PHAR control should be inherently integrative—which is to say, not to solely focused on PHAR eradication or native revegetation results as mutually exclusive but assessing simultaneously when applicable. Evidence for this assertion was discussed in the literature review at length, where most common, traditional methods reviewed have generally had mercurial outcomes on both sides of the spectrum (failure/success) (Wilson et al., 2004). Combined treatments using cultural methods in addition to the more intensive methods discussed in this study (i.e., solarization, herbicide and mowing) may provide a greater understanding of the complex dynamics involved in the restoration of PHAR dominated wetlands over time.

Given PHAR impingements on wetland restoration efforts and its demonstrated impact on the OSF, it is imperative to advance research on innovative treatment methods to control this species. A better understanding of how integrated restoration techniques affect PHAR under varying hydrology and changing climate conditions, as well as their long-term effects on vegetation structure and composition could significantly benefit restoration efforts in the Pacific Northwest. Regarding OSF habitat conservation specifically, additional research into herbicidal and cultural methods appear to be the most promising avenue for future studies seeking to improve the efficiency of restoration designs for seasonally inundated, PHAR dominated wetlands in the Pacific Northwest.

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# Appendices

# Appendix A

## **Additional Species Lists**

## Table A 1

## Extraneous Non-target Species List (2019 Monitoring)

Ctr	Scientific Name	Species Code	Standard English Name		Manipulated Target
1	Bromus hordeaceus	BRHO	Soft brome (also termed Soft cheat or Soft chess)	Exotic	No
2	Carex obnupta	CAOB	Slough sedge	Native	No
3	Carex utriculata	CAUT	Common beaked sedge (also termed Swollen beaked sedge)	Native	No
4	Cirsium arvense	CIAR	Creeping thistle (also known as Canada thistle)	Exotic	No
5	Cirsium vulgare	CIVU	Bull thistle (also termed as Common or Spear thistle)	Exotic	No
6	Eleocharis palustris	ELPA	Common spike-rush	Native	Yes
7	Equisetum arvense	EQAR	Common horsetail (also termed Field horsetail)	Native	No
8	Galium aparine	GAAP	Goosegrass (also termed Cleavers or Catchweed)	Exotic	No
9	Galium triflorum	GATR	Sweet Bedstraw (also termed Fragrant Bedstraw)	Native	No
10	Geranium molle	GEMO	Dove's-foot Crane's-bill (also termed Dovefoot Geranium)	Exotic	No
11	Glyceria elata	GLEL	Fowl mannagrass	Native	Yes
12	Hypericum perforatum	HYPE	St. John's Wort (also termed Common St. John's Wort)	Exotic	No
13	Juncus effusus	JUEF	Common rush	Native	No
14	Juncus supiniformis	JUSU	Spreading rush (also termed Hair-leaved rush)	Native	Yes
15	Lotus corniculatus	LOCO	Bird's-foot trefoil	Exotic	No
16	Myosotis micrantha	MYMI	Small-flowered forget-me-not	Native	No
17	Myosotis scorpioides	MYSC	True forget-me-not (also termed Water forget-me-not)	Native	No
18	Phalaris arundinacea	PHAR	Reed canarygrass	Exotic	Yes
19	Plantago major	PLMA	Broadleaf plantain (also termed Greater plantain)	Exotic	No
20	Poa unknown	POA	Bluegrass species	Exotic	No
21	Pseudognaphalium stramineum	PSST	Cotton-batting-plant (also termed Cotton-batting cudweed)	Native	No
22	Ranunculus repens	RARE	Creeping buttercup	Exotic	No
23	Rubus armeniaca	RUAR	Himalayan blackberry (also termed Armenian blackberry)	Exotic	No
24	Senecio jacobea	SEJA	Common ragwort (also termed Tansy ragwort)	Exotic	No
25	Solidago canadensis	SOCA	Canada goldenrod	Native	No
26	Spirea douglasii	SPDO	Hardhack (also termed Western Spirea or Steeple Bush)	Native	No
27	Trifolium dubium	TRDU	Lesser trefoil (also termed Suckling clover)	Exotic	No
28	Veronica americana	VEAM	American brooklime (also termed American speedwell)	Native	No
29	Veronica beccabunga	VEBE	European speedwell (also termed Brooklime)	Exotic	No

# Appendix B

#### Metadata

## Table B 1 Monitoring and Study Design Metadata

Variable	Description
Site	One of three study locations: Mima Creek, Watkins, and West Rocky Prairie
Depth	One of three inundation depths: Shallow (S), Moderate (M), and Deep (D) (3 inundation depths exist at each site [= study location])
Array	One of three arrays: 1, 2, and 3 (3 arrays exist for every inundation depth)
Trt	One of two treatments: Control (C), Vegetation Mats (VM)
Plot	One of four plots: 1, 2, 3, and 4
%GLEL	Percentage of Glyceria elata (GLEL)
%JUSU	Percentage of Juncus supiniformis (JUSU)
%ELPA	Percentage of <i>Eleocharis palustris</i> (ELPA)
% TotalN	Percentage of Total Natives (this is simply the sum of %GLEL, %JUSU, & %ELPA)
%PHAR	Percentage of <i>Phalaris arundinea</i> (PHAR)
%GLEL	Percentage of Glyceria elata (GLEL)
%JUSU	Percentage of Juncus supiniformis (JUSU)
%ELPA	Percentage of <i>Eleocharis palustris</i> (ELPA)
% TotalN	Percentage of Total Natives (this is simply the sum of %GLEL, %JUSU, & %ELPA)
%PHAR	Percentage of <i>Phalaris arundinea</i> (PHAR)
%GLEL	Percentage of Glyceria elata (GLEL)
%JUSU	Percentage of Juncus supiniformis (JUSU)
%ELPA	Percentage of <i>Eleocharis palustris</i> (ELPA)
% TotalN	Percentage of Total Natives (this is simply the sum of %GLEL, %JUSU, & %ELPA)
%PHAR	Percentage of <i>Phalaris arundinea</i> (PHAR)
Thatch	Thatch depth (cm) - This is the depth of the rhizome thatch beneath the above-ground plant structure
Native Richness	Number of native species
N-Native Richness	Number of non-native species

Note. Terminology for water depth/hydrology/zone has been changed to "Inundation depths" in

this table. Colors are to help distinguish categories of variables.

# Appendix C

## Additional 2019 Statistics Material

Original Scoring			Colla	ipsed Sc	oring	Analysis Original Scoring Scoring			apsed		
Scored	Scored Bin Interval		Scored	Bin Interval		Scored	B in Interval		Scored	Bin Interval	
Value	Lower	Upper	Value	Lower	Upper	Value	Lower	Upper	Value	Lower	Upper
0	0	0	0	0	0	0.5	0	1	0.5	0.0001	1
0.5	0.0001	1	0.5	0.0001	1	3	2	5	3	2	5
3	2	5	3	2	5	8	6	10	8	6	10
8	6	10	8	6	10	15	11	20	30	11	50
15	11	20	30	11	50	25	21	30	75	51	100
25	21	30	75	51	100	35	31	40			
35	31	40				45	41	50			
45	41	50				55	51	50			
55	51	50				65	61	60			
65	61	60				75	71	80			
75	71	80				85	81	90			
85	81	90				95	91	100			
95	91	100									

Table	<b>C</b> 1	Original	(2017-2018)	and Collapsed	(2019)	Vegetation	Monitoring	Scoring
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*Note.* Table showing cover class interval adjustments made for the beta regression model.

					lower	upper
Year	Depth	Site	mu	Sigma	95% HPD	95% HPD
2017	D	Mima Creek	0.0648	0.0192	0.0311	0.1018
2018	D	Mima Creek	0.5669	0.0570	0.4542	0.6783
2019	D	Mima Creek	0.9340	0.0209	0.8920	0.9718
2017	М	Mima Creek	0.0825	0.0247	0.0399	0.1294
2018	М	Mima Creek	0.5496	0.0578	0.4382	0.6554
2019	М	Mima Creek	0.9266	0.0232	0.8806	0.9667
2017	S	Mima Creek	0.2068	0.0449	0.1217	0.2953
2018	S	Mima Creek	0.8467	0.0356	0.7711	0.9121
2019	S	Mima Creek	0.9637	0.0119	0.9424	0.9851
2017	D	Watkins	0.0246	0.0079	0.0111	0.0403
2018	D	Watkins	0.0395	0.0095	0.0210	0.0579
2019	D	Watkins	0.0608	0.0164	0.0317	0.0947
2017	М	Watkins	0.0222	0.0078	0.0091	0.0372
2018	М	Watkins	0.0307	0.0080	0.0167	0.0456
2019	М	Watkins	0.0804	0.0214	0.0445	0.1238
2017	S	Watkins	0.0263	0.0086	0.0115	0.0427
2018	S	Watkins	0.0496	0.0125	0.0266	0.0744
2019	S	Watkins	0.0851	0.0236	0.0439	0.1328
2017	D	West Rocky	0.0269	0.0084	0.0127	0.0449
2018	D	West Rocky	0.2083	0.0382	0.1425	0.2903
2019	D	West Rocky	0.6508	0.0555	0.5363	0.7506
2017	М	West Rocky	0.0526	0.0162	0.0248	0.0859
2018	М	West Rocky	0.3427	0.0481	0.2504	0.4325
2019	М	West Rocky	0.7944	0.0437	0.7079	0.8785
2017	S	West Rocky	0.0388	0.0125	0.0153	0.0631
2018	S	West Rocky	0.1942	0.0358	0.1253	0.2662
2019	S	West Rocky	0.5239	0.0582	0.4145	0.6429

**Table C 2**. Depth by Site Treatment Beta RegressionOutput Table

*Note*: HPD = highest posterior density intervals.

# Equation 1

Logistic regression assessment point model (APM)

APM : 
$$log it [P(y_{i,t} = 1)] = y_0 + y_1 Yr_t$$
.

Note. Taken from Irvine and Rodhouse (2010).