Revegetation of Bulrushes Bolboschoenus Maritimus, Schoenoplectus Acutus, and S. Americanus in Great Salt Lake Wetlands: Seed Biology and Influence of Environmental Factors on Rhizomes

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REVEGETATION OF BULRUSHES BOLBOSCHOENUS MARITIMUS, SCHOENOPLECTUS ACUTUS, AND S. AMERICANUS IN GREAT SALT LAKE WETLANDS: SEED BIOLOGY AND INFLUENCE OF ENVIRONMENTAL FACTORS ON RHIZOMES

by

James Marty

A thesis submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

Ecology

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2016
ABSTRACT

Revegetation of Bulrushes *Bolboschoenus maritimus*, *Schoenoplectus acutus*, and *S. americanus* in Great Salt Lake Wetlands: Seed Biology and Influence of Environmental Factors on Rhizomes

by

James Marty, Master of Science
Utah State University, 2016

Major Professor: Dr. Karin M. Kettenring
Department: Watershed Sciences

A primary goal of ecological restoration is to establish desirable plant species. This goal is particularly important following the removal of invasive plants. Understanding biological traits of plant species important to revegetation is crucial to plant establishment. In the globally important Great Salt Lake (GSL) wetlands, native habitat-forming bulrushes *Bolboschoenus maritimus*, *Schoenoplectus acutus*, and *S. americanus* are frequently displaced by the invasive grass *Phragmites australis*. Successful revegetation of bulrushes relies on improving our understanding of seed dormancy break, seed germination requirements, and the environmental factors affecting rhizome emergence and growth. We used a series of germination chamber and greenhouse experiments to examine effective seed dormancy break treatments and germination conditions for multiple collection sites of bulrushes *B. maritimus*, *S. acutus*, and *S. americanus*. We also performed a greenhouse experiment to investigate how water
depth, nutrient, and salinity levels affect *B. maritimus* and *S. acutus* emergence and growth from rhizomes. Cold, moist stratification and bleach scarification were effective dormancy break treatments for all species, though magnitude of effect varied by species and source site. Soaking the seeds after application of dormancy break treatments improved germination for all species. Rhizome emergence of *S. acutus* was negatively affected by high water depth, likely due to oxygen limitation. *Bolboschoenus maritimus* was salinity tolerant relative to *S. acutus*. GSL wetland managers can use these findings to improve revegetation projects via seeding and planting.

(85 pages)
Revegetation of Bulrushes *Bolboschoenus maritimus*, *Schoenoplectus acutus*, and *S. americanus* in Great Salt Lake Wetlands: Seed Biology and Influence of Environmental Factors on Rhizomes

James Marty

A primary goal of ecological restoration is to establish desirable plant species. This goal is particularly important following the removal of invasive plants. Understanding biological characteristics of plant species important to revegetation is crucial to plant establishment. In the globally important Great Salt Lake (GSL) wetlands, native habitat-forming bulrushes *Bolboschoenus maritimus*, *Schoenoplectus acutus*, and *S. americanus* are frequently displaced by the invasive grass *Phragmites australis*.

Bulrush seeds require very specific germination conditions and are also dormant, therefore they do not germinate consistently even when exposed to appropriate germination conditions. Additionally, wetland environments are often associated with water level fluctuations, high salinities, and nutrient loading, which may affect bulrush establishment from planted material such as rhizomes. Successful revegetation of bulrushes relies on improving our understanding of seed dormancy break, seed germination requirements, and the environmental factors affecting rhizome emergence and growth. We used a series of germination chamber and greenhouse experiments to examine effective seed dormancy break treatments and germination conditions for multiple collection sites of bulrushes *B. maritimus*, *S. acutus*, and *S. americanus*. We also performed a greenhouse experiment to investigate how water depth, nutrient, and salinity
levels affect B. maritimus and S. acutus emergence and growth from rhizomes. Cold, moist storage and bleach treatments were effective dormancy break treatments for all species, though the effect varied by species and site. Soaking the seeds in water after application of dormancy break treatments improved germination for all species. Rhizome emergence of S. acutus was negatively affected by high water depth, likely due to oxygen limitation. Bolboschoenus maritimus was salinity tolerant relative to S. acutus. GSL wetland managers can use these findings to improve revegetation projects via seeding and planting.

James Marty
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CHAPTER 1
INTRODUCTION

Wetlands provide important ecosystem services such as wildlife habitat, flood control, and water filtration that are reduced or eliminated due to wetland destruction and degradation (Zedler and Kercher 2005). Loss of the ecosystem services provisioned by wetlands necessitates ecological restoration. To ensure plant establishment in wetland revegetation projects, it is critical to improve the knowledge of basic biological information of species and processes that are pertinent to restoration (Clewell and Rieger 1997; Young et al. 2005; Cabin et al. 2010). The understanding of basic biological information for many habitat-forming wetland plants that are important to restoration remains incomplete. Poor understanding of seed dormancy break, seed germination, and rhizome growth requirements hinders the establishment of restored plant populations. In these studies, we evaluate factors affecting revegetation of perennial bulrushes Bolboschoenus maritimus, Schoenoplectus acutus, and S. americanus in the globally important Great Salt Lake (GSL) wetlands in Utah, USA.

In GSL wetlands, there is a crucial need to understand the seed dormancy break and germination requirements as well as environmental conditions affecting emergence and growth of native bulrushes B. maritimus, S. acutus, and S. americanus. Within the GSL watershed, all three species form large monospecific stands and serve as important habitat and food sources for migratory birds on the Pacific and Central Flyways. Bulrush seeds and rhizomes provide a primary nutrition supply for waterfowl, and bulrush vegetation offers cover and nesting habitat. The non-native grass Phragmites australis has invaded GSL wetlands and is estimated to cover 10,000 hectares, replacing stands of
native vegetation such as *B. maritimus*, *S. acutus*, and *S. americanus* (Kettenring et al. 2013). Extensive treatment programs have been implemented to remove *P. australis*, but there is poor recruitment of native species (Kettenring et al. 2014). Managers of GSL wetlands have pursued reestablishment of *B. maritimus*, *S. acutus*, and *S. americanus* via seeds, but seeding efforts have been unsuccessful due to a deficiency of basic information on seed traits (Chad Cranney, Randy Berger, Rich Hansen, UT Division of Wildlife Resources, pers. comm.). Vegetative propagules such as sod mats and plugs were successful for one dike revegetation project, but unpublished data indicates results may not be applicable to whole management unit revegetation projects due to fluctuations in water levels and salinity (Chad Cranney, pers. comm.; England et. al., unpublished data). An improved understanding of seed dormancy break and germination requirements in addition to the environmental factors that affect rhizome growth is needed.

Seeding is a desirable option for many revegetation projects, as seeds are relatively inexpensive compared to vegetative propagules and efficient to apply on large scales (100-1000 km$^2$) (Kettenring and Galatowitsch 2007; Palmerlee and Young 2010; Merritt and Dixon 2011). Seeding is cheap and convenient to apply, but seedling establishment can often be lower than 10% due to a failure to break dormancy or to sow into adequate germination conditions (Budelsky and Galatowitsch 2004; Merritt and Dixon 2011). Many species require particular environmental conditions (e.g., moist-cold treatment that mimics winter conditions) to break seed dormancy and if treatments are not applied, germination fails even when germination conditions are appropriate. Even if dormancy break is achieved, it can be challenging to provide adequate germination conditions, particularly in wetlands, where unpredictable fluctuations in water levels can
change moisture conditions rapidly. Therefore, it is essential to develop the understanding of seed dormancy break and germination for specific species to improve revegetation efforts via seed.

Understanding how basic environmental factors affect rhizomes is important before implementation of large-scale revegetation efforts. Environmental factors such as water-depth fluctuations, salinity levels, and nutrient enrichment present significant challenges to the establishment of wetland graminoids from rhizomes (Yetka and Galatowitsch 1998; Galatowitsch et al. 1999; Budelsky and Galatowitsch 2000). Growth of rhizomes may only occur under narrow ranges of environmental conditions, and it is important to understand the response of rhizomes to these abiotic factors.

My research addressed the following questions to investigate factors driving regeneration of *B. maritimus*, *S. acutus*, and *S. americanus* by seeds and rhizomes in GSL wetlands: 1) What are the most effective dormancy break treatment and germination conditions for *B. maritimus*, *S. acutus*, and *S. americanus*, and does response to dormancy break and germination conditions vary by source site? (Chapter 2) 2) How do water depth, nutrient, and salinity levels affect emergence and growth of *B. maritimus* and *S. acutus*? (Chapter 3).

References


CHAPTER 2
GUIDANCE FOR THE SEED DORMANCY BREAK AND GERMINATION OF THREE GLOBALLY IMPORTANT WETLAND BULRUSHES: BOLBOSCHOENUS MARITIMUS, SCHOENOPLECTUS ACUTUS, AND S. AMERICANUS

Abstract

Plant establishment is a primary goal of ecological restoration that requires an understanding of biological traits for plant species important to revegetation. Limited knowledge of seed dormancy break and germination requirements hinders revegetation of habitat-forming species Bolboschoenus maritimus, Schoenoplectus acutus, and S. americanus. Through a series of germination chamber and greenhouse experiments we provide guidance for effective dormancy break and seed germination conditions for these species. For S. acutus, 180 day cold, moist stratification was the most effective dormancy break treatment in one experiment, but 30 day stratification improved germination comparably in two other experiments. Longer stratification lengths for S. acutus may result in more consistently high germination percentages, but 30 day stratification may be of sufficient length for some source sites. For S. americanus, stratification improved germination in two of three experiments, but 20-60% of viable seed did not germinate across all experiments, potentially due to a poor understanding of germination requirements. Soaking seeds in water improved the germination of all species when applied after dormancy break treatments. Importantly, response to dormancy break

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1 Co-authored with Karin M. Kettenring, Bret Mossman, and Delena Williams.
treatment varied among source sites, and intraspecific variation may account for unpredictable responses to dormancy break treatments. Taken together, these findings can assist practitioners in crafting strategies to effectively break dormancy and germinate seeds of *B. maritimus*, *S. acutus*, and *S. americanus*.

Key words: *Bolboschoenus maritimus*, *Schoenoplectus*, seed dormancy, seed germination, intraspecific variation

**Introduction**

Ecological restoration is critical for reversing ecosystem degradation and loss. Research on biological information for species and processes relevant to restoration is crucial to ensure successful plant establishment in revegetation projects (Clewell and Rieger 1997, Young et al. 2005, Cabin et al. 2010). Yet for many dominant, habitat-forming plants that are the target of restoration, such data are lacking. Active revegetation of plant species via seed is an attractive option due to the relatively low cost of seed compared to vegetative propagules and the need to perform revegetation on large scales (100-1000 km²) (Kettenring and Galatowitsch 2007a, Palmerlee and Young 2010, Merritt and Dixon 2011). However, seed traits such as requirements for dormancy break and germination can greatly limit propagation and revegetation success for plant species targeted for restoration efforts (Hoag and Sellers 1995, Merritt and Dixon 2011). In this study we focus on the seed dormancy and germination of *Bolboschoenus maritimus*, *Schoenoplectus acutus*, and *S. americanus*, three globally distributed bulrushes critical for wildlife habitat and the target of restoration efforts. Based on results of our research and
previous studies with these species, we develop guidance for practitioners to successfully break dormancy and germinate seeds of *B. maritimus*, *S. acutus*, and *S. americanus*.

*Bolboschoenus maritimus*, *S. acutus*, and *S. americanus* are perennial graminoids of the family Cyperaceae that are habitat-forming species across their global ranges. All three species form large monospecific stands that provide critically important food and habitat for millions of migratory birds on the Pacific and Central Flyways of North America (Pederson and Pederson 1983, Hohman et al. 1990, Olson et al. 2004). Though commonly thought to reproduce primarily asexually, recent research in the Great Salt Lake (GSL) Basin, the location of the present study, documented that *B. maritimus* has high levels of genetic diversity (measured as genet and allelic richness) at small spatial scales and small clone size, indicators of substantial spread by seed (Sweetman et al. 2013). A recent unpublished study on genetic structuring of *S. acutus* and *S. americanus* in this region found at least moderate genetic diversity (measured as genet richness) even at 1m² spatial scales, evidence that reproduction is not limited to vegetative means (K. Kettenring et al., Utah State University, unpub. data).

In GSL wetlands, there is a crucial need to understand the seed dormancy break and germination requirements of native bulrushes *B. maritimus*, *S. acutus*, and *S. americanus*. The invasive grass *Phragmites australis* is estimated to cover 10,000 hectares of GSL wetlands, displacing native vegetation such as *B. maritimus*, *S. acutus*, and *S. americanus* (Kettenring et al. 2013). Thousands of hectares of *P. australis* have been or are being treated, but native species are not returning (Kettenring et al. 2014). There is great interest in reestablishing *B. maritimus*, *S. acutus*, and *S. americanus* via seeding, but to date seeding has failed due to lack of basic information on seed dormancy break and
germination requirements (C. Cranney, R. Berger, R. Hansen, UT Division of Wildlife Resources, pers. comm.). Seeds for restoration of *B. maritimus*, *S. acutus*, and *S. americanus* are relatively abundant due to naturally high seed production outputs per stem and intensive collection efforts, but seeding efforts have largely been unsuccessful. It is unclear whether recent failures of manager seeding efforts were due to low rates of dormancy break or failure to sow seeds into appropriate germination conditions. Growers produce plugs that establish with higher efficiency, but even under controlled greenhouse conditions emergence can be limited due to spatial and temporal variation in viability and germinability between seed lots (J. Klausmann, North Fork Native Plants, pers. comm.). Further, large-scale planting of vegetative propagules in GSL wetlands is not economically feasible, since revegetation programs and needs are on the scale of thousands of hectares.

The seed biology of plants in the family Cyperaceae is complex, and divergent seed traits exist even among closely related species (Schütz and Leck 2005, Kettenring and Galatowitsch 2007b). These species have low-risk germination strategies that are well suited to forming persistent seed banks and taking advantage of infrequent canopy gaps (Schütz and Leck 2005). These strategies involve very specific dormancy break and germination requirements. Thus, it is extremely difficult to determine straightforward propagation protocols restoration of these species. A number of previous studies have focused on seed traits of *B. maritimus*, *S. acutus*, and *S. americanus* (Table 1), but these studies yielded conflicting findings regarding dormancy break and germination requirements. These discrepancies may be due to inter-annual variation in seed lot requirements (Gutterman 2000, Kettenring and Galatowitsch 2007b), and/or intraspecific
variation among source sites (Thullen and Roberts 1995, Moriera et al. 2012; Kettenring 2015, in review). Our study addresses these issues by comparing protocols recommended in previous work and considering different and multiple populations within the study region to provide direction for dormancy break and germination of bulrush seed in the Intermountain West.

**Previous research on seed dormancy break in the study species**

All Cyperaceae with seed dormancy exhibit physiological dormancy (Leck and Schütz 2005, Baskin and Baskin 2014). Though physiologically dormant, some Cyperaceae seeds possess thick waxy coats that readily imbibe water, but have embryos with such low growth potential that physical scarification may be necessary for the radicle to emerge (Baskin and Baskin 2014). For *B. maritimus*, the USDA Plant Guide recommends 30 days of cold, moist stratification, but it is unclear whether these recommendations are based on internal research or peer-reviewed studies (Tilley 2012a). However, 28-120 day stratification has been demonstrated to be less effective for European *B. maritimus* and ineffective for GSL Basin populations of *B. maritimus* (Clevering 1995, Fraissé et al. 1997, Kettenring, in review). In contrast, 240 day stratification improved European *B. maritimus* dormancy break, and longer stratification times have not been examined for North American populations (Clevering 1995). Relatively long-term stratification may relieve physiological dormancy while simultaneously breaking down the seed coat to allow the embryo to overcome low growth potential (Clevering 1995). Chemical scarification improves germination of *B. maritimus* relative to short-term stratification (Clevering 1995, Kettenring, in review),
though a North American study still failed to germinate 25-30% of viable seeds (Kettenring, in review). The USDA Plant Guide for *S. acutus*, suggests 75 days of pre-chilling, but again it is unclear whether these recommendations are based on internal research or peer-reviewed studies (Tilley 2012b). For two populations of Colorado *S. acutus*, germination improved for 84 day stratification compared to shorter stratification lengths, supporting the USDA recommendation, though the response to stratification length varied between source sites (Thullen and Eberts 1995). Conclusions about *S. acutus* response to dormancy break are based on few populations, and multiple other studies showed germination failure in >20% of viable seed (Isely 1944, Kaushik 1963, Klausmann 1998, Kellogg et al. 2003). Very little is known regarding *S. americanus* dormancy break. The Native Plant Network propagation protocol cites only one study of a single collection site from over 70 years ago, and no other study directly examines dormancy break (Isely 1944).

Further consideration must also be given to seed after-ripening, a period of storage where freshly harvested seeds are kept dry at room temperature for a period of time in which complex molecular pathways can affect depth of dormancy (Finch-Savage and Leubner-Metzger 2006). Published studies have not consistently documented the effect of after-ripening on *B. maritimus*, *S. acutus*, or *S. americanus*. Dry after-ripening improved germination of three European *Carex* spp. and is known to decrease depth of dormancy in a number of plant species (Schütz 1997, Probert 2000). Seeds used in one of the most successful *B. maritimus* studies were stored dry at 4 °C for one and five years (Clevering 1995). All other studies were performed on seeds dry after-ripened for less than one year (Table 1).
It is also possible that a combination of scarification and stratification is necessary to break dormancy. Endozoochory by ducks has been proposed as a primary mode of dispersal for these species and therefore a requirement for the combined effects of scarification and stratification on dormancy is ecologically plausible (Mueller and van der Valk 2002, Figuerola et al. 2010). Duck passage can improve germination of *B. maritimus* compared to untreated controls (Wongsriphuek et al. 2008). The combination of mechanical scarification in the gizzard and chemical scarification in the digestive tract could weaken the seed coat enough so that the embryo can emerge. Some growers of these species have indicated that a combination of chemical scarification and long-term cold, moist stratification is the best technique for breaking seed dormancy (R. Mandel, Golder Associates, pers. comm.). Further evaluation of these combined scarification and stratification effects is warranted. Given the dearth of studies on seed dormancy break in *B. maritimus*, *S. acutus*, and *S. americanus*, and because those studies were based off of few populations, there is a crucial need to study dormancy break to guide restoration protocols for these bulrush species in the Intermountain West region.

*Previous research on germination requirements for the study species*

Most Cyperaceae seeds respond well to high, fluctuating summer temperatures with ample light levels, and this appears true for *B. maritimus* and *S. acutus* (Clevering 1995, Thullen and Eberts 1995, Leck and Schütz 2005, Kettenring, in review). Flooding has a positive effect on *B. maritimus* germination, though the effect of flooding is unknown for *S. acutus* and *S. americanus* (Clevering 1995, Kettenring, in review). *Schoenoplectus acutus* germinates well in moist conditions (Thullen and Eberts 1995).
The USDA plant guide for *S. americanus* suggests placing seeds in 3 cm of standing water for germination (Favorite 2002). Soaking seeds of some Cyperaceae in warm water is useful for greenhouse propagation and pre-germination before field planting (Tilley 2013; Tilley and St. John 2013). Under field conditions, it is important to know whether flooding is necessary for germination, as it is extremely difficult to field-sow seeds into standing water without wave action displacing them and depositing them on the shoreline. There remains a need to further examine the moisture requirements of *B. maritimus*, *S. acutus*, and *S. americanus* so that adequate germination conditions are established for propagation and field establishment.

In order to evaluate *B. maritimus*, *S. acutus*, and *S. americanus* dormancy break and germination requirements, we asked the following questions: 1) What is the effect of stratification length and dry after-ripening on dormancy break and germination of *B. maritimus*, *S. acutus*, and *S. americanus* (Experiment 1)? 2) Do combinations of cold stratification and physical scarification improve seed dormancy break of *B. maritimus*, *S. acutus*, and *S. americanus* (Experiments 2 and 3)? 3) What is the effect of moisture level and soaking on *B. maritimus*, *S. acutus*, and *S. americanus* germination (Experiments 1 and 3)? 4) Does response to dormancy break treatments and germination conditions vary among seed collection sites (Experiments 1 and 3)?

**Methods**

*Experiment 1: Effect of site, seed dry after-ripening, short-term and long-term cold stratification, and moisture levels on bulrush dormancy loss and germination*

The objective of this experiment was to determine whether cold, moist stratification relative to dry after-ripening has an effect on *B. maritimus*, *S. acutus*, and *S. americanus* dormancy loss.
*Americanus* dormancy loss and germination, and whether response to these treatments varies by collection site. A secondary objective of this experiment was to determine whether moisture level has an effect on germination.

For *B. maritimus* and *S. acutus*, we collected seed from four wetland source populations in Utah and Idaho in fall 2014 (Figure 1). All seed was hand collected from at least three discrete patches ≥ 100 m² in size and ≥ 100 m apart. Due to the low seed production of *S. americanus*, we were unable to collect our own seed in sufficient quantities and instead used seed donated by the Utah Division of Wildlife Resources collected from multiple unidentified patches in southeastern Box Elder County, Utah in fall 2014. We assessed seed viability by performing tetrazolium assays (TZ concentration = 0.1%) on three replicates of one hundred seeds for each site. Seeds of all species were stored dry at room temperature (approximately 21°C) for 6 months prior to experiment. At the start of the experiment, the seeds were 180 days old.

Each treatment combination was replicated twice in two separate chambers. Each replicate consisted of a Petri dish of at least 50 seeds. We placed seeds in Petri dishes filled with white silica sand. We cold-stratified seeds by wrapping samples in paint strainers, burying them in a sand/peat moss mixture, and placing them in a cold room at 4°C. Seeds were cold-stratified for 30 or 180 days. Germination rates were then compared with untreated, dry after-ripened seeds at time zero, and untreated, dry after-ripened seeds 30 and 180 days after the experiment started. The stratification treatments were chosen based on previous literature and conversations with native plant growers who suggested that longer stratification may improve *Bolboschoenus* and *Schoenoplectus* spp. germination rates (Clevering 1995).
We placed the Petri dishes in Conviron CMP 3244 growth chambers set to 35°C /18°C corresponding to a 12:12 hour photoperiod with a photosynthetic photon flux (PPF) set to 1500 μmol m⁻² s⁻¹. We chose these temperature and light regimes based on previous research and pilot studies indicating that high light levels combined with these average summer temperatures representative of GSL wetland conditions support high germination in these species (Clevering 1995, Kettenring, in review). Our moisture regime treatments were saturated or flooded conditions. In the saturation treatment, Petri dishes were watered until surface pooling of water. Samples undergoing the flooding treatment were watered to 0.5 cm of water above the substrate surface. Samples were watered twice daily to the prescribed levels. Seed germination was recorded weekly for 4 weeks.

We used a generalized linear mixed model with a binomial distribution and a logit link to examine the fixed effects of stratification/dry after-ripening (5 levels), moisture regime (2 levels), and site (4 levels) on germination percentage. The design was a randomized complete block: sample dishes were randomly assigned to combinations of the three fixed effects factors and nested within growth chambers. Chamber was included in the model as a fixed effects blocking factor due to the small number of chambers (n=2) and estimation problems. Estimation problems also required that the three-way interaction term be omitted from the model. The model included the interaction of chamber and fixed effects factors to address overdispersion. Calculations were made using the GLIMMIX procedure in SAS/STAT 14.1 in the SAS System for Windows 9.4 TS1M3.
Experiment 2: Effect of cold stratification, chemical scarification, and mechanical scarification on bulrush seed dormancy loss among source populations

The objective of this experiment was to determine whether 30 day stratification, chemical scarification using bleach and hydrochloric acid, mechanical scarification, and interactions between these seed treatments affects dormancy loss and germination of *B. maritimus*, *S. acutus*, and *S. americanus*. The scarification treatments were chosen based on previous literature (Baskin and Baskin 1971, Clevering 1995, Milotić and Hoffman 2015), conversations with native plant growers, and pilot studies that found that seeds extracted from duck gizzards germinated to high percentages (Mossman and Williams, unpub. data).

The seed source, seed collection, seed viability, and seed storage methods were the same as used in section Experiment 1. Seed age at initiation of the experiment was ~210 days. For *B. maritimus* and *S. acutus*, only seeds from Salt Creek WMA (Figure 1) were used because they were the most germinable in pilot studies.

Seeds were cold-stratified using the same methods described for Experiment 1. We bleach-scarified seeds using household bleach diluted to 3% sodium hypochlorite. Seed samples were wrapped in paint strainers and placed in 3% bleach for either 24 or 48 hours. Acid scarified samples were placed in concentrated 37.4% hydrochloric acid for either 30 or 40 minutes. After each chemical scarification, seeds were rinsed thoroughly with deionized water. Mechanical scarification was performed by placing seeds in a box lined with 120 grit sandpaper and rubbing seeds between a block coated with sand paper and the box. No additional force was applied by the operator to prevent unintended variation in operator force. Low treated seeds were scarified for 15 seconds and high
treated seeds were scarified for 30 seconds. For samples recieving a combination of treatments, the treatments followed the following order of application: chemical scarification then mechanical scarification then cold stratification.

Each treatment combination was replicated three times in all three species. Each replicate was at least 100 seeds. Samples were randomly placed in growth chambers within a greenhouse. The greenhouse was maintained at an average of daytime 35°C : nighttime 28°C with supplemental lighting to provide a 14 hour/10 hour photoperiod between 800 and 1600 m$^{-2}$ s$^{-1}$. Each growth chamber was constructed of a 15.14 L clear plastic wash bins with three aluminum tins (11 cm x 22 cm) placed inside. Each tin was filled with 800 mL of white silica sand and divided into four sections, with a seed sample placed in each of the four sections. The growth chambers were then watered so that tins were sub-irrigated to saturation. We punctured the bottom of the tins to allow movement of water upward through the sand. The growth chambers were covered with clear plastic wrap to increase humidity and minimize surface drying since samples were being sub-irrigated. We measured temperature within the chambers prior to the experiment to ensure that their temperatures were not greater than ambient greenhouse temperature. The clear bins were maintained at approximately 2000 mL of water to allow for consistent saturation within the tins. Germination was recorded weekly for 5 weeks. The experimental design was inadequate to appropriately analyze the data, as we failed to record the distribution of treatment combinations within tins (random blocks). Therefore, we report solely descriptive results.
Experiment 3: Effect of site, cold stratification, chemical scarification, and soaking on bulrush seed dormancy loss and germination

The objective of this experiment was to investigate the effect of different sites, sulfuric acid scarification, and a 14 day soak in deionized water on dormancy break and germination of *B. maritimus*, *S. acutus*, and *S. americanus*. The treatments were chosen for evaluation because 1) differences in response to dormancy loss treatments can vary by population, 2) pilot studies and conversations with native plant growers suggested sulfuric acid may be more effective than hydrochloric acid at breaking seed dormancy, and 3) a previous study suggested that soaking is important to *Bolboschoenus* and *Schoenoplectus* spp. germination (Tilley 2013). Our stratification treatments were no stratification and 30 day stratification. Our chemical treatments were no chemical scarification, a 48 hour soak in 3% bleach, and a 40 minute soak in 2.67% H$_2$SO$_4$. Our soaking treatments were no soak and a 14 day soak. The seed source, seed collection, seed viability, and seed storage methods were the same as described in Experiment 1. Seed age at the initiation of the experiment was ~ 360 days. *B. maritimus* and *S. acutus* sites included in this experiment were Salt Creek WMA and Farmington Bay WMA (Figure 1).

Seeds were stratified using the same methods described in Experiment 1. Bleach-scarified samples were placed in 3% bleach for 48 hours and scarification was performed using the same methods described in Experiment 2. Acid scarified samples were placed in 2.67% H$_2$SO$_4$ for 40 minutes. We soaked seed samples by placing samples in cups of deionized water for 2 weeks before transfer to growth chambers. We changed water for the soaking treatment every three days. For samples requiring a combination of
treatments, the treatments followed the following order of application: chemical scarification then cold stratification then the water soak.

The experimental design was identical to the design described in Experiment 2 except for the important difference that the distribution of treatment combinations within tins (random blocks) was recorded making analysis using incomplete random blocks possible. Seed maintenance and data collection were identical to the methods described in section 2.2. We used a generalized linear mixed model with a binomial distribution and a logit link to examine the fixed effects of stratification (2 levels), chemical scarification (3 levels), soaking (2 levels), and site (2 levels) on germination percentage. Aluminum tin was included in the model as a random blocking effect. Due to low replication and estimation problems, we omitted the three- and four-way interaction terms from the model. *S. americanus* seeds came from only one site; because the small data set posed estimation problems, we omitted the random block effect from the model, in addition to the fixed-site effect. For all analyses, calculations were made using the GLIMMIX procedure in SAS/STAT 14.1 in the SAS System for Windows 9.4 TS1M3.

**Results**

Seed viability was high for all species, although the ST site was less viable than the other sites for both *B. maritimus* and *S. acutus* (Table 2).

*Experiment 1: Effect of site, seed dry after-ripening, short-term and long-term cold stratification, and moisture levels on bulrush dormancy loss and germination*

Stratification, site, and moisture had significant effects on germination percentage of *B. maritimus* (Table 3). The highest germination percentages were for seeds with a 180
day stratification treatment (Figure 2). Germination percentage was improved to a lesser extent in seeds stratified for 30 days and for the 180 day untreated seeds (i.e., seeds dry after-ripened for 360 days). For the 180 day stratification treatment, FB (86% ± 3%; mean ± standard error) and SC (80% ± 3%) had the highest germination percentage, and ST (67% ± 3%) had a higher germination percentage than BL (27% ± 5%). Germination percentage was higher for flooded seeds (22% ± 2%) than for saturated (18% ± 3%) seeds.

Stratification and site had significant effects on germination percentage of *S. acutus* (Table 3). The highest germination percentages were for seeds with a 180 day stratification treatment (75% ± 3%) (Figure 2). Across all treatments FB (57% ± 4%) and SC (61% ± 3%) seeds had higher germination percentage than BL (43% ± 4%), while BL had a higher germination percent than ST (27% ± 3%).

There were no significant differences among treatments for *S. americanus* (Table 3). All seed, regardless of treatment, germinated from 50-77% (Figure 2).

**Experiment 2: Effect of cold stratification, chemical scarification and mechanical scarification on bulrush seed dormancy loss among source populations**

*Bolboschoenus maritimus* seed germination was highest with either one or two days of bleach scarification (44-82%) regardless of other treatments (Figure 3). Thirty day stratification also improved germination of seeds that were not bleach-scarified. HCl scarification appeared to weakly improve seed germination of unstratified seeds, but it did not improve germination of stratified seeds.
Schoenoplectus acutus seed germination was highest with 30 day stratification (66-83%), regardless of other treatments (Figure 3). No other treatment had a discernible effect on germination percentage.

Schoenoplectus americanus seed germination was highest with 30 day stratification and bleach scarification (60% ± 3%) (Figure 3). Thirty day stratification improved germination percentage by 32% regardless of other treatments.

Experiment 3: Effect of site, cold stratification, chemical scarification, and soaking on bulrush seed dormancy loss and germination

Stratification, scarification, soaking, and site had significant effects on germination percentage of B. maritimus (Table 4; Figure 4). Germination percentage was consistently highest with bleach scarification (49-74%), regardless of whether seeds were stratified. Soaking for 14 days increased germination percentage compared to unsoaked seeds by 13%. For no scarification or H₂SO₄ scarification, 30 day stratification improved germination percentage. For non-stratified seeds, germination percentage was higher at SC (33% ± 7%) than FB (24 ± 7%); with stratification, germination percentage increased and did not differ between sites.

Stratification, scarification, soaking, and site had significant effects on germination percentage for S. acutus (Table 4; Figure 4). Germination percentage did not change with stratification for bleach-scarified seeds but stratification improved germination percentage by 56% for seeds that were not scarified or were H₂SO₄ scarified. Seeds that were not scarified or H₂SO₄ scarified and stratified germinated to the highest percentages (80-89%). Soaking improved germination for stratified seeds by 18% but did not improve germination of unstratified seeds. Germination percentage was high and
similar for soaked seeds regardless of scarification treatment; seeds that were not soaked had lower germination percentage when seeds were either not scarified or were bleach-scarified. There was no difference in germination percentage between sites for bleach-scarified seeds; germination percentage was higher for SC (55% ± 5%) than FB (43% ± 6%) for seeds that were either H₂SO₄ scarified or not scarified.

Stratification and scarification had significant effects on germination percentage for *S. americanus* (Table 4; Figure 4). Stratification increased germination percentage for all scarification treatments. In the absence of stratification, bleach-scarified seeds had higher germination than seeds that were H₂SO₄ scarified or not scarified. Scarification had no effect on stratified seeds.

**Discussion**

In this study, we document important responses to seed dormancy break treatments and germination conditions for three widely distributed, habitat-forming bulrushes, *B. maritimus*, *S. acutus*, and *S. americanus*. Seeds of these species generally responded well to cold, moist stratification as a dormancy break treatment, though response to length of stratification varied among species. Additionally, bleach scarification as a dormancy breaking treatment also improved germination, though again the magnitude of the response varied by species. Soaking seeds in water for 14 days improved seed germination, particularly when used in combination with dormancy breaking treatments. We also found that the response to stratification varied among sites for *B. maritimus* and *S. acutus*. These dormancy break treatments and germination
conditions can be used to improve establishment of these bulrush species in wetland restoration applications.

**Dormancy break treatments**

*Bolboschoenus maritimus*

Cold, moist stratification for 180 days was the most effective treatment for *B. maritimus* achieving >80% germination for SC and FB sites and >65% for the ST site, thereby likely germinating most of the viable seeds. Bleach scarification also was a very effective dormancy breaking treatment, with >60% of seeds germinating in each of the two scarification experiments. Also of note is that 30 day stratification and dry after-ripening improved seed germination, though not to the extent that 180 day stratification or bleach scarification did. Our results follow a similar pattern to previous results from Europe that demonstrated 240 day stratification and bleach scarification as more effective than 28 and 42 day stratification (Table 1) (Clevering 1995). The effectiveness of 180 day stratification and bleach scarification indicate that the embryo may have low growth potential that is not sufficient to break open the seed coat. Both long-term stratification and bleach scarification likely break down the seed coat, allowing the embryo to push through (LaCroix and Mosher 1995, Schütz and Leck 2005, Baskin and Baskin 2014). Numerous variables need to be considered when deciding if long-term stratification or bleach scarification is the preferred dormancy breaking treatment for *B. maritimus*.

In this study, long-term stratification resulted in the germination percentage. However, the germination trials in Experiment 1 were performed in tightly controlled growth chambers in comparison to the germination trials following the bleach
scarification treatments (Experiments 2 and 3), which were performed in a greenhouse where the environment was controlled but exposed to more variation. Comparisons of long-term stratification and bleach scarification should be made under the same environmental conditions. Additionally, dry after-ripening could be a mitigating variable in these studies. We found that untreated seeds dry after-ripened an additional 180 days (360 total days of dry after-ripening) germinated at similar rates to seeds stratified for 30 days. Two previous studies have found that dry after-ripening did not affect germination percentage for *B. maritimus*. However, one of these studies (Kettenring, in review) did not use seeds dry after-ripened for more than 180 days. The other study (Clevering 1995) used much older seeds (420-1,980 days) and stored seeds at 4°C instead of room temperature, though Clevering (1995) found that older seeds required more specific germination conditions. For many species, after-ripening releases seed dormancy (Finch-Savage and Leubner-Metzger 2006). The effect of dry after-ripening for *B. maritimus* remains unclear, though the results from this study suggest using fresh seed that has not been dry after-ripened may be undesirable for maximizing seed germination for restoration applications.

From a restoration perspective, long-term stratification potentially requires years of planning for seed source and seed quantity and such time may not be available on a restoration project timeline. However, other studies find that *B. maritimus* seeds stratified for 240, 560, and 1,800 days all germinated to very high percentages (Clevering 1995). It is likely that *B. maritimus* seed may remain highly germinable for long periods of cold stratification. Practitioners may be able to place seeds lots in permanent stratification until ready to use so that highly germinable seed is consistently available. In contrast,
bleach scarification takes only days to apply, but results in lower germination compared to cold-stratified seeds. Many bleach-scarified seeds that failed to germinate seemed to be damaged by the bleach; perhaps if bleach concentrations and soak times could be refined, fewer seeds would be damaged and higher germination percentages would be achieved. Until more data are available, practitioners must weigh the advantages of both long-term stratification and bleach scarification, knowing that each is an effective dormancy breaking technique.

*Schoenoplectus acutus*

Stratification resulted in the highest germination percentages for *S. acutus*, though the response varied by experiment. In Experiment 1, 180 day stratification was extremely effective and 30 day stratification did not differ from two of three controls. However, in Experiments 2 and 3, 30 day stratification was extremely effective, equaling germination percentages observed for 180 day stratification in Experiment 1. Previous studies exhibited a similar pattern to Experiment 1. Seed from sites in Colorado germinated to >85% after 84 days stratification compared to 40-60% germination percent for 28 day cold stratification (Thullen and Eberts 1995). We did not examine stratification lengths between 30 and 180 days, but the success of Thullen and Eberts (1995) suggest intermediate stratification lengths could break dormancy for some sites. As with *B. maritimus*, dry after-ripening and intraspecific variation could be mitigating factors that explain differences in response to stratification lengths among our experiments.

Bleach scarification is an effective treatment for seeds that were not stratified, though the positive effect was not very large. Stratification length >30 days is the
preferred dormancy break treatment, though 30 day stratification may be sufficient for some seeds. However, if time is an issue, bleach scarification may be an efficient option for practitioners.

*Schoenoplectus americanus*

Though 30 day stratification mildly and moderately improved germination in Experiments 2 and 3 for *S. americanus* (Figures 3 and 4), there were no differences among stratification and control treatments in Experiment 1 (Figure 2). Bleach scarification may also mildly improve germination, but results were inconsistent between the two greenhouse experiments. Dormancy break treatments for *S. americanus* only mildly improve germination and 20-60% of viable seed remained ungerminated throughout all experiments. More research on *S. americanus* seed germination and dormancy break is needed in order to determine the most effective method to germinate the remaining viable seed, particularly by examining more sites and a wider range of germination conditions. We were only able to use one site for *S. americanus*, and it is possible that this particular site has a higher dormancy depth relative to other sites (similar to BL in the Experiment 1 for *B. maritimus*). Further, there are no peer reviewed studies that examine germination conditions for *S. americanus*, and it is possible that in our study seeds were non-dormant and failed to germinate due to inadequate germination conditions.

*Effect of moisture and soaking*

Soaking seeds in water before placing them in germination conditions had a positive effect on germination of *B. maritimus*. For *S. acutus*, soaking only had a positive
effect on seeds that were stratified, and for *S. americanus* soaking had a marginally significant positive effect. In Experiment 1 (Figure 2) there was a positive effect of flooding seeds for *B. maritimus*. Flooding or soaking improves germination for numerous aquatic species, including *B. maritimus* (Clevering 1995, Tilley 2013, Baskin and Baskin 2014, Kettenring, in review). Seeds exposed to flooded or soaking conditions may become fully imbibed more quickly, thus leading to faster germination (Baskin and Baskin 2014). Soaking may lower oxygen concentrations which can improve seed germination of some Cyperaceae (Pons and Schröder 1986). In contrast, soaking with aerated water improved germination of another Cyperaceae plant relative to soaking without aeration, and may provide both oxygen and fully submerged conditions that improve germination (Tilley 2013). We changed water every three days for our soak treatment, which may provide an intermediate level of aeration (Tilley 2013). Future research could examine a range of oxygen concentrations within soak treatments to determine the effect of aeration. Soaking is a technique that may be useful to growers germinating large quantities of seed, as it is easy to place large quantities of seed into standing water in contrast to trying to provide more precise environmental conditions. However, providing flooded conditions in a restoration site is problematic as seeds can wash away via wave action or flow and accumulate on banks. Worse, if seeds germinate in flooded conditions, seedlings are vulnerable to flooding stress and high mortality is possible (Seabloom et al. 1998, Budelsky and Galatowitsch 2004, Tilley and St. John 2013). Therefore, we recommend soaking only as a useful tool for greenhouse propagation and pre-germination prior to field sowing into saturated or moist conditions.
**Intraspecific variation among sites**

We found intraspecific variation in depth of dormancy for both species (*B. maritimus* and *S. acutus*) where multiple collection sites were examined. Differences in seed germination among sites for *S. acutus* in Experiment 1 are likely due to differences in seed viability (Experiment 1; Table 2; Figure 2). The other differences for *B. maritimus* and *S. acutus* are likely due to variation in depth of dormancy and consistent with other studies demonstrating intraspecific variation of seed traits for these species (Experiments 1 and 3; Table 1; Figures 2 and 4) (Thullen and Roberts 1995, Kettenring in review).

Mechanisms of seed dormancy are very complex and generally poorly understood, particularly at a species level, making it extremely difficult to parse the causes of variation in dormancy (Bewley 1997, Fincher-Savage and Leubner-Metzger 2006). Physiological dormancy is regulated by molecular processes, which in turn are regulated by environmental conditions of the maternal plant during seed development and of the seed germination microsite. For *B. maritimus* and *S. acutus*, understanding of dormancy is further complicated by embryos that lack the physical strength to push through the seed coat. Variation in depth of dormancy for these species could be due to differing molecular level responses to environmental conditions or physical barriers resulting from a thicker seed coat. We were unable to discern a mechanism causing variation in depth of dormancy nor were we able to determine if variation was due to maternal effects or genetic effects. High genetic diversity (allelic richness and clonal diversity) of *B. maritimus* exists in the region and variation in depth of dormancy and germination requirements has been previously documented for this species (Sweetman et
Preliminary studies indicate at least moderate genetic diversity at small spatial scales for *S. acutus* and *S. americanus* (K. Kettenring et al., Utah State University, unpub. data). Moderate to high genetic diversity in these species allows the possibility that variation in depth of dormancy has a genetic basis. The sites used in this study vary in climate and hydrologic management and could result in strong selection pressures leading to distinct adaptations affecting physiological dormancy or seed coat thickness. Environmental conditions, particularly hydrology, could also result in strong maternal effects on depth of dormancy. Reciprocal transplant studies focused on discerning genetic and maternal effects may help explain the source of intraspecific variation.

**Conclusions and implications for restoration**

Results of this study provide strategies for breaking dormancy and improving germination of *B. maritimus*, *S. acutus*, and *S. americanus*, three bulrush species that have presented a challenge to researchers and practitioners. Long-term (180 days) cold, moist stratification and bleach scarification are both excellent tools for breaking dormancy of *B. maritimus*. Cold, moist stratification of any length of time will improve germination of *S. acutus*, though longer stratification (>30 days) may be better. Dormancy break requirements for *S. americanus* remain unclear, though cold stratification improves germination, and failed germination of viable *S. americanus* seed may be due to a poor understanding of environmental conditions required for germination in the present study. Soaking seeds of all species after application of dormancy break
treatments improves germination and is a useful technique for germinating large quantities of seed.

Our findings exhibit similar patterns of response to dormancy break treatments for *B. maritimus* and *S. acutus* as in previous studies (Table 1). However, it is clear from both our study and comparisons among previous studies that intraspecific variation exists that may result in variation in response to dormancy break treatments. Practitioners must integrate the expectation of site variation in response to dormancy break into seeding strategies.

**Acknowledgments**

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Isely, D. 1944. A study of conditions that affect the germination of Scirpus seed. Cornell University Agricultural Experiment Station Memo 257:1-8.


### Table 1. Methods and results from studies examining dormancy break and germination of *B. maritimus*, *S. acutus*, and *S. americanus*.

<table>
<thead>
<tr>
<th>Study</th>
<th>Maximum germination percentage (treatment)</th>
<th>Treatments</th>
<th>Seed age (days)</th>
<th>Germination conditions</th>
<th>Populations collected (location)</th>
<th>Notes</th>
</tr>
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<tbody>
<tr>
<td>(Clevering 1995)</td>
<td>98% (2 day soak in 4% bleach)</td>
<td>14, 28, 42, 240, 560, 1800 day cold moist stratification; bleach scarification</td>
<td>420; 730; 1980</td>
<td>Incubator; 12 h photoperiod; 30°C/5°C; 0.5 cm water</td>
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<td>(Fraissé et al. 1997)</td>
<td>6% (120 day cold moist stratification)</td>
<td>90, 120 day room temperature soak; 90, 120 day cold moist stratification; mechanical scarification</td>
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<td>(Kaushik 1963)</td>
<td>85% (no pre-treatment)</td>
<td>salinity (4 levels); frozen in ice</td>
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<td>Incubator; dark; 35°C; moist</td>
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<td>Methods unclear; low replication; Some degree of intraspecific variation in response to dormancy break treatment</td>
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<td>(Kettenring, in review)</td>
<td>75% (1 day soak in 3% bleach)</td>
<td>30, 90 day cold moist stratification; bleach scarification</td>
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<td>Growth chambers; light; 35°C/18°C; moist</td>
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Table 1. (cont.)

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<td>Winter photoperiod (Ithaca, NY); 30-32°C; moisture conditions not specified</td>
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<td>Low replication; seed collected from few plants; seed age not consistent, just differently aged seed lots</td>
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<td>----------------</td>
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<td>----------------------</td>
<td>---------------------</td>
<td>-------</td>
<td></td>
</tr>
<tr>
<td>(Weiher and Keddy 1995)</td>
<td>83% (not specified)</td>
<td>Not specified</td>
<td>Incubator; 12 h photoperiod; 25°C/15°C</td>
<td>Not specified</td>
<td>USDA plant guide; does not cite quantitative data or peer-reviewed research</td>
<td></td>
</tr>
<tr>
<td>(Favorite 2002)</td>
<td>Not specified</td>
<td>Not specified</td>
<td>Not specified</td>
<td>3 cm of water in a cold frame</td>
<td>Low replication; seed collected from few plants; seed age not consistent, just differently aged seed lots</td>
<td></td>
</tr>
<tr>
<td>(Isely 1944)</td>
<td>67% (180 day cold moist stratification)</td>
<td>90 day cold wet stratification; 180 day cold moist stratification; 180 day frozen in ice</td>
<td>Winter photoperiod (Ithaca, NY); 30-32°C; moisture conditions not specified</td>
<td>Fresh; 540; 900</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Keddy and Constabel 1986)</td>
<td>20% (150 day cold moist stratification)</td>
<td>150 day cold moist stratification</td>
<td>Surface sown; greenhouse conditions; saturated moisture</td>
<td>155</td>
<td>2 (Ontario)</td>
<td></td>
</tr>
<tr>
<td>(Keddy and Ellis 1985)</td>
<td>50% (30 day cold moist stratification)</td>
<td>30 day cold moist stratification</td>
<td>Surface sown; greenhouse conditions; -1 cm moisture</td>
<td>40</td>
<td>2 (Ontario)</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Seed viability for *B. maritimus*, *S. acutus*, and *S. americanus*.

<table>
<thead>
<tr>
<th>Population</th>
<th>Percent viable seed (mean ± 1 SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>B. maritimus</strong></td>
<td></td>
</tr>
<tr>
<td>BL</td>
<td>96 ± 1</td>
</tr>
<tr>
<td>FB</td>
<td>96 ± 1</td>
</tr>
<tr>
<td>SC</td>
<td>95 ± 2</td>
</tr>
<tr>
<td>ST</td>
<td>62 ± 13</td>
</tr>
<tr>
<td><strong>S. acutus</strong></td>
<td></td>
</tr>
<tr>
<td>BL</td>
<td>86 ± 3</td>
</tr>
<tr>
<td>FB</td>
<td>98 ± 1</td>
</tr>
<tr>
<td>SC</td>
<td>93 ± 4</td>
</tr>
<tr>
<td>ST</td>
<td>59 ± 2</td>
</tr>
<tr>
<td><strong>S. americanus</strong></td>
<td></td>
</tr>
<tr>
<td>Box Elder County</td>
<td>96 ± 2</td>
</tr>
</tbody>
</table>
Table 3. The effects of cold stratification/dry after-ripening, moisture regime, and site on seed germination of *B. maritimus*, *S. acutus*, and *S. americanus*.

<table>
<thead>
<tr>
<th>Effect</th>
<th>DF</th>
<th>F-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>B. maritimus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stratification</td>
<td>4, 51</td>
<td>90.39</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Site</td>
<td>3, 51</td>
<td>24.71</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Moisture</td>
<td>1, 51</td>
<td>5.13</td>
<td>0.03</td>
</tr>
<tr>
<td>Stratification*site</td>
<td>12, 51</td>
<td>0.99</td>
<td>0.47</td>
</tr>
<tr>
<td>Stratification*moisture</td>
<td>4, 51</td>
<td>0.48</td>
<td>0.75</td>
</tr>
<tr>
<td>Site*moisture</td>
<td>3, 51</td>
<td>0.73</td>
<td>0.54</td>
</tr>
<tr>
<td><strong>S. acutus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stratification</td>
<td>4, 39</td>
<td>23.40</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Site</td>
<td>3, 39</td>
<td>24.76</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Moisture</td>
<td>1, 39</td>
<td>1.27</td>
<td>0.27</td>
</tr>
<tr>
<td>Stratification*site</td>
<td>12, 39</td>
<td>0.56</td>
<td>0.86</td>
</tr>
<tr>
<td>Stratification*moisture</td>
<td>4, 39</td>
<td>0.65</td>
<td>0.63</td>
</tr>
<tr>
<td>Site*moisture</td>
<td>3, 39</td>
<td>0.50</td>
<td>0.69</td>
</tr>
<tr>
<td>Stratification<em>site</em>moisture</td>
<td>12, 39</td>
<td>0.56</td>
<td>0.86</td>
</tr>
<tr>
<td><strong>S. americanus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stratification</td>
<td>4, 9</td>
<td>1.23</td>
<td>0.36</td>
</tr>
<tr>
<td>Moisture</td>
<td>1, 9</td>
<td>1.46</td>
<td>0.26</td>
</tr>
<tr>
<td>Stratification*moisture</td>
<td>4, 9</td>
<td>0.60</td>
<td>0.67</td>
</tr>
</tbody>
</table>
Table 4. The effects of stratification, chemical scarification, soaking, and site on *B. maritimus* and *S. acutus* seed germination, and the effects of stratification, chemical scarification, and soaking on *S. americanus* seed germination.

<table>
<thead>
<tr>
<th>Effect</th>
<th>DF</th>
<th>F-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>B. maritimus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stratification</td>
<td>1, 22</td>
<td>113.72</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Scarification</td>
<td>2, 22</td>
<td>95.31</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Soaking</td>
<td>1, 22</td>
<td>32.13</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Site</td>
<td>1, 22</td>
<td>7.60</td>
<td>0.01</td>
</tr>
<tr>
<td>Stratification*site</td>
<td>1, 22</td>
<td>8.76</td>
<td>0.01</td>
</tr>
<tr>
<td>Stratification*scarification</td>
<td>2, 22</td>
<td>46.99</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Stratification*soaking</td>
<td>1, 22</td>
<td>2.09</td>
<td>0.16</td>
</tr>
<tr>
<td>Scarification*soaking</td>
<td>2, 22</td>
<td>1.50</td>
<td>0.24</td>
</tr>
<tr>
<td>Soaking*site</td>
<td>1, 22</td>
<td>0.37</td>
<td>0.55</td>
</tr>
<tr>
<td>Scarification*site</td>
<td>2, 22</td>
<td>0.83</td>
<td>0.45</td>
</tr>
<tr>
<td><strong>S. acutus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stratification</td>
<td>1, 18</td>
<td>556.50</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Scarification</td>
<td>2, 18</td>
<td>7.45</td>
<td>0.00</td>
</tr>
<tr>
<td>Site</td>
<td>1, 18</td>
<td>20.73</td>
<td>0.00</td>
</tr>
<tr>
<td>Soaking</td>
<td>1, 18</td>
<td>16.61</td>
<td>0.00</td>
</tr>
<tr>
<td>Stratification*site</td>
<td>1, 18</td>
<td>1.34</td>
<td>0.26</td>
</tr>
<tr>
<td>Stratification*scarification</td>
<td>2, 18</td>
<td>134.82</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Stratification*soaking</td>
<td>1, 18</td>
<td>36.24</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Scarification*soaking</td>
<td>2, 18</td>
<td>9.40</td>
<td>0.00</td>
</tr>
<tr>
<td>Scarification*site</td>
<td>2, 18</td>
<td>8.68</td>
<td>0.00</td>
</tr>
<tr>
<td>Soaking*site</td>
<td>1, 18</td>
<td>3.56</td>
<td>0.08</td>
</tr>
<tr>
<td><strong>S. americanus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stratification</td>
<td>1, 25</td>
<td>82.12</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Scarification</td>
<td>2, 25</td>
<td>9.04</td>
<td>0.01</td>
</tr>
<tr>
<td>Soaking</td>
<td>1, 25</td>
<td>3.38</td>
<td>0.08</td>
</tr>
<tr>
<td>Stratification*scarification</td>
<td>2, 25</td>
<td>8.27</td>
<td>0.00</td>
</tr>
<tr>
<td>Stratification*soaking</td>
<td>1, 25</td>
<td>0.33</td>
<td>0.57</td>
</tr>
<tr>
<td>Scarification*soaking</td>
<td>2, 25</td>
<td>0.23</td>
<td>0.80</td>
</tr>
</tbody>
</table>
Fig. 1. Seed collection sites in ID and UT. We collected seeds of *B. maritimus* and *S. acutus* from Bear Lake National Wildlife Refuge, ID (BL), Farmington Bay Waterfowl Management Area, UT (FB), Salt Creek Waterfowl Management Area, UT (SC), and Sterling Wildlife Management Area, ID (ST). Seeds of *S. americanus* were collected in Box Elder County, UT (see legend).
Fig. 2. The effects of cold stratification, moisture regime, and site on seed germination of *S. acutus*, *S. americanus*, and *B. maritimus*. Means and standard errors were computed from the raw data. Seeds of *S. americanus* were collected from a single site and therefore *S. americanus* data are presented in a separate legend.
Fig. 3. The effects of 30 day cold stratification, chemical scarification, and mechanical scarification on seed germination of *S. acutus*, *S. americanus*, and *B. maritimus*. Means and standard errors were computed from the raw data.
Fig. 4. The effects of 30 day cold stratification, chemical scarification, soaking, and site on seed germination of *S. acutus*, *S. americanus*, and *B. maritimus*. Means and standard errors were computed from the raw data. Seeds of *S. americanus* were collected from a single site and therefore *S. americanus* data are presented in a separate legend.
CHAPTER 3

COLLABORATING WITH JAILS FOR EFFECTIVE AND EFFICIENT BIOLOGICAL RESEARCH: RESULTS FROM A GREENHOUSE EXPERIMENT ON ABIOTIC DRIVERS OF EMERGENCE AND GROWTH OF TWO BULRUSH SPECIES

ABSTRACT

Recovery of the ecosystem services associated with wetlands requires revegetation of habitat-forming plant species such as *Bolboschoenus maritimus* and *Schoenoplectus acutus*. A poor understanding of the basic biological traits associated with these species is a challenge for revegetation efforts. In this study we address how water-depth, nutrients, and salinity levels affect rhizome establishment of *B. maritimus* and *S. acutus*. Additionally, we engage Salt Lake County Jail in collaborative research to perform effective and efficient research that engages the jail population. To our knowledge, this is the first research project completed in a jail setting. High water-depth negatively affected *S. acutus* and we found *B. maritimus* to be more salinity tolerant than *S. acutus*. These results can inform wetland revegetation and management strategies. Inmates who participated responded positively to involvement with the project, and we concluded that collaboration between researchers and jails can produce mutually beneficial outcomes.

Index terms: alkali bulrush, hardstem bulrush, sustainability in prisons project, revegetation, rhizome
INTRODUCTION

Wetlands provide important ecosystem services such as wildlife habitat, flood control, and water filtration that are reduced or eliminated due to wetland destruction and degradation (Zedler and Kercher 2005). Due to their low elevation within landscapes, wetlands are especially vulnerable to plant invasions (Zedler and Kercher 2004), which requires improved wetland revegetation. Many wetland restorations involve removal of invasive plants, but often do not include active revegetation. Wetland plants, particularly the foundational perennial graminoids, rarely recolonize due to habitat fragmentation and dispersal limitations (Pfadenhauer and Grootjans 1999; Mulhouse and Galatowitsch 2003; Kettenring and Galatowitsch 2011). Revegetation of these species by seeding and planting is necessary to restore functioning wetland plant communities. Establishing wetland graminoids is difficult due to environmental constraints (particularly water-level fluctuations) and inadequate knowledge of the biological traits that lead to high survivorship (Budelsky and Galatowitsch 2000; Galatowitsch et al. 1999; Yetka and Galatowitsch 1998). Understanding how basic environmental factors affect revegetation is important before implementation of large-scale revegetation efforts. In this study, we evaluate factors affecting revegetation of perennial graminoids in the continentally important Great Salt Lake (GSL) wetlands in Utah, USA.

Native *Bolboschoenus maritimus* L. (Palla) (alkali bulrush) and *Schoenoplectus acutus* (Muhl. Ex Bigelow) Á. Löve & D. Löve (hardstem bulrush) of GSL wetlands provide globally important habitat for migratory shorebirds and waterfowl of the Pacific and Central Flyways. *Bolboschoenus maritimus* and *S. acutus* are widely distributed, perennial wetland graminoids of the family Cyperaceae found in freshwater and brackish
wetlands across North America (Ball et al. 2002). Within GSL wetlands, *B. maritimus* and *S. acutus* form large monospecific stands. Both species are considered excellent waterbird habitat due to abundant seed and tuber production and vegetative cover (Evans and Martinson 2008). Waterfowl feed on the nutritious bulrush seeds and take cover and nest in the bulrush vegetation. However, along the GSL, stands of bulrush species in human-impacted wetlands are commonly replaced by the invasive grass *Phragmites australis* (Kettenring et al. 2012). Thousands of hectares of *P. australis* have been or are being treated, but native species are not returning through natural processes (Kettenring et al. 2014). The few revegetation projects implemented have not been successful (Chad Cranney, Randy Berger, UT Division of Wildlife Resources, *pers. comm.*). Greenhouse-propagation and field-planting protocols exist for each species, and they suggest that with proper control of water-levels, high establishment can be achieved (Hoag et al. 2001). However, GSL wetlands present unique challenges to revegetation including high salinities, unpredictable hydrologic regimes, and high nutrient loading (Downard et al. 2014; Downard, unpublished data).

Additionally, it is frequently necessary in restoration ecology to conduct relatively straightforward, even simple, experiments (such as this one) to gather essential biological information (hereafter referred to as “basic”) to guide restorations or inform more ecologically sophisticated or larger experiments. Such basic biological information can be difficult to find due to a dearth of peer-reviewed studies or because such information is often presented in difficult-to-find “gray literature”. Further, basic biological information may not be directly applicable to the species or region of interest due to the trait diversity within plant families with closely related species (e.g., Cyperaceae) and
ecotypic variation. Conducting basic biological research is often resource intensive. Purchasing or collecting plant materials, plant maintenance, experimental setup, and facility space are expensive yet necessary to develop basic studies. However, such research endeavors can be difficult to fund due to their lack of novelty. Fostering research approaches that investigate basic biological information while expending minimal resources may help to alleviate this dilemma for researchers.

A novel approach, collaboration between researchers and incarcerated populations, is a promising opportunity that is gaining recognition in conservation and restoration science. The U.S. incarcerated population comprises over 2.2 million adults, a subset of which participate in educational, therapeutic, or employment-related programs while incarcerated (Glaze and Herberman 2013). The introduction of conservation and research programs in Washington, Oregon, Maryland, and Ohio as part of the national Sustainability in Prisons Project (SPP) Network, has been viewed as a win-win for all involved (Kaye et al. 2015). Institutions within the SPP Network have engaged prison inmates in native-plant production, rearing and research of state and federally listed endangered species, sustainability projects (e.g. gardening, recycling) and research projects on moss cultivation (Kaye et al. 2015; Conlon et al. 2013; Aubrey 2013; Ulrich and Nadkarni 2009). Some of these activities are accompanied by lectures and workshops for inmates. Exposing inmates to conservation and research presents numerous benefits including intellectual stimulation from lectures and workshops, therapeutic value from plant maintenance, a connection to the community and environment, and post-release educational opportunities (Lindemuth 2014; Ulrich and Nadkarni 2009; Cho and Tyler 2013; Tyler and Kling 2006). For researchers, prisons provide facilities and a unique
opportunity to work with a diverse population eager to participate in research and
cconservation projects, making science in prisons an excellent fit for resource-intensive
basic biological studies.

However, programs in the SPP Network have yet to attempt restoration research
projects in jails. Jails are typically run by sheriffs or local governments and hold
individuals with relatively short stays (< 1 year, usually 1-3 months), in contrast to
prisons, in which individuals carry out longer-term sentences. Jails present new
challenges to SPP Network programs, as the inmate-turnover rate makes it more difficult
to train and retain inmates for long-term participation in research projects. Although
education programs are known to reduce the risk of recidivism and increase the odds of
post-release employment (Davis et al. 2013), there are generally fewer education program
opportunities in jails compared to prisons (Harlow 2003). Therefore, science education
opportunities through involvement in restoration research can provide important
enrichment for the jail population.

In this study, we examine the environmental factors that drive *B. maritimus* and *S.
acutus* regeneration from rhizomes. Our primary objective was to determine how water-
depth, nutrient, and salinity levels affect *S. maritimus* and *S. acutus* emergence and
growth from rhizomes. Our secondary objective of this study was to evaluate jails as a
venue for conducting effective and efficient basic biological research that engages and
benefits the jail inmate population. Previous work has described case studies and models
for involving prison inmates in conservation and restoration activities. Here we focus on
implementing experiments within a jail system as a feasible approach to generate basic
biological information on wetland plant species and to guide restoration efforts in addition to the benefits these activities provide to inmates (Kaye et al. 2015).

METHODS

Collaboration with the INSPIRE Program and the Salt Lake County Jail

Salt Lake County Jail (SLCJ) inmates (hereafter “trustees”) participated in this project as part of the INSPIRE (Initiative to Bring Science Programs to the Incarcerated) Program. The INSPIRE Program at the University of Utah is a part of the SPP Network and promotes science education at correctional institutions in Utah. INSPIRE provides a monthly lecture series to the SLCJ and Draper Prison and initiated a jail conservation project focused on captive rearing of a state sensitive fish species. SLCJ trustees that participated in our project were minimum-security inmates with good behavior, and either volunteered or were selected for the program by jail officers. Trustees were also a part of the SLCJ’s horticulture program that grew ornamental plants and produce. SLCJ has two existing greenhouses affiliated with the horticulture program; one greenhouse was partially vacant during the winter and was made available for this short-term research project.

The trustees assisted in the experiment setup and maintenance and daily data collection for this research project. Officers and horticulture staff provided additional supervision and coordination, particularly when researchers and INSPIRE staff were not present. USU researchers and/or INSPIRE staff made weekly visits to provide guidance and monitor progress, but the trustees took ownership of the daily plant care and data collection in between the weekly visits. We tracked trustee involvement through
attendance records and plant-care data sheets, and we collected feedback through exit surveys that used multiple-choice, open-ended, and Likert-scale questions. Surveys were administered to active participants at the conclusion of the project. Given the high degree of trustee turnover, this approximates the spectrum of involvement we would expect at any point in time on the project. We provided six workshops over the course of the project on topics focused on the GSL ecosystem, wetland plant ecology, and wetland restoration to provide more context to the trustees on the importance of their involvement in this research.

**Experimental Design**

Using a fully factorial experimental design with 5 replicates of each treatment combination, we determined the effect of water-depth, nutrients, salinity, and their interactions on *B. maritimus* and *S. acutus* rhizome emergence, growth, and productivity in a greenhouse experiment from January 31 until July 23, 2014. Rhizomes were subjected to two water-depth treatments (saturated or inundated), two nutrient treatments (high or low), and two salinity treatments (high or low). The treatments were applied to mesocosms of *B. maritimus* and *S. acutus* in the SLCJ greenhouse facilities.

**Mesocosms**

In November 2013, we collected *B. maritimus* and *S. acutus* rhizomes from the U.S. Fish and Wildlife Service’s Bear River Migratory Bird Refuge (BR) and the Utah Division of Wildlife Resources’ Farmington Bay Waterfowl Management Area (FB), two wetland areas on the GSL. Rhizomes were placed in cold storage for 45 days to induce dormancy until planting (McIntinch and Garbisch 2004). Before planting, we trimmed
rhizomes to a consistent size (three buds) and measured the length and mass of each rhizome for use as a covariate in our statistical models. We planted rhizomes in a substrate mix of Turface Quick Dry calcined clay and Alaskan peat moss at a 2:1 (v:v) ratio. We planted one rhizome from each site into every pot to increase the probability of having plant emergence. The first emerging rhizome was maintained for the experiment while the other rhizome was then removed. We mixed in GSL wetland soil collected from Farmington Bay at a 1% volume ratio to introduce microbial communities to the root zone (Green and Galatowitsch 2000). We used 150-L pots without drains to provide a closed basin to allow manipulation of water-levels.

**Treatment Application**

We selected a high water-depth treatment of 10 cm to provide a treatment where rhizomes would be in a shallowly flooded, anaerobic environment, similar to field conditions where the species are known to persist. We selected a low water-depth treatment of -5 cm (5 cm below the substrate surface) to provide a moist substrate. Pots were watered daily to the prescribed water-depth treatment. Water-depth was measured below the substrate surface via a perforated PVC pipe fixed in the substrate that was equilibrated with the pot water-level fluctuations. Water-level beneath the soil surface was visible through the PVC pipe and marked by the top of a wooden dowel.

We selected nutrient levels based on total plant available nitrogen (ammonium and nitrate) levels measured in GSL wetlands (Rebekah Downard, Utah State University, *unpubl. data*). The low nutrient treatment was 1.58 g N/L and the high nutrient treatment
was 6.33 g N/L. We applied Jack’s Professional 20-10-20 (% N-P-K) Peat-Lite liquid fertilizer every two weeks at the prescribed levels.

We selected salinity levels based on salinities measured in Great Salt Lake wetlands (Rebekah Downard, Utah State University, unpubl. data). The low salinity treatment was untreated culinary water measured at 0.2 ppt and the high salinity treatment was 6 ppt. The high salinity treatment was achieved by making a single addition of Instant Ocean Sea Salt at a rate of 7 g/L at the beginning of the experiment. We monitored salinities weekly using a VEE GEE STX-3 Salinity Scale Optical Refractometer.

**Vegetation Data Collection**

We measured plant height and stem density weekly. Due to plant die-offs near the end of the experiment, we used peak plant height (tallest plant height during the length of the experiment) in our statistical analysis. We harvested above and belowground biomass at the end of the experiment at 22 weeks. Biomass samples were cleaned and dried at 80°C for 24 hours, and the dry weight was recorded.

**Vegetation Data Analysis**

We performed a 3-way completely randomized design Analysis of Variance (ANOVA) for *B. maritimus* in which plant height, stem density, aboveground biomass, belowground biomass, and total biomass were response values (separate ANOVAs) and water-depth, nutrients, and salinity were fixed effects. Due to low emergence of *S. acutus*, the sample size was inadequate for ANOV. Therefore, results are summarized solely with descriptive statistics. We performed a Pearson’s Chi-squared test to determine
if environmental factors affected the probability *S. acutus* emergence (high emergence of *B. maritimus* made Chi-squared analysis unnecessary).

**RESULTS**

**Bulrush Response to Flooding, Salinity, and Fertilization**

For *B. maritimus*, there was a significant positive effect of high nutrients on stem density, belowground biomass, and total biomass (Table 1; Figures 1 & Supplemental Figure 1). *Bolboschoenus maritimus* plant height was highest in response to high nutrients and low salinity. *Schoenoplectus acutus* plant height, stem density, and biomass measurements were all highest in response to low salinity (Figures 1 & Supplemental Figure 1). Emergence of *S. acutus* was greater with low water-depth ($\chi^2 = 4.912; p < 0.05$).

**Trustee Involvement in Research Project and Response to Enrichment Activities**

Fifty-one different male trustees worked on the project over the course of six months. Trustees were incarcerated for a variety of misdemeanors and felonies. On average, each trustee worked on the project for 4.6 weeks and provided 6.5 hours of total labor (320 hours of total labor). Additionally, USU researchers and INSPIRE staff together provided another 160 hours of labor for trustee training and data-collection activities. Due to the relatively short length of stay for inmates at the jail and the fact that the supplemental GSL lectures were scheduled 2-4 weeks apart, only 32 of the 51 trustees were able to participate in one or more lectures.

Approximately 25% of the trustees (13) completed an exit survey at the conclusion of the program, and only 2 of these trustees had been involved with the
project since the beginning. Of those, 85% (11) of survey respondents reported that they received some or all of their training on the project from another trustee, and 62% (8) reported training another trustee. Sixty-nine percent (9) somewhat agreed or strongly agreed that they received enough training, while 23% (3) neither agreed nor disagreed, and 8% (1) somewhat disagreed. Additionally, 85% (11) responded that they somewhat agreed or strongly agreed that they understood the purpose of the experiment, learned “a lot” about plant science, and learned “a lot’ about general science by participating.

Seventy-seven percent (10) strongly agreed that the project made them think about continuing their education (2 respondents neither agreed nor disagreed, and 1 did not answer). Seventy-seven (10) strongly agreed that they “enjoyed participating in the program”, while the remaining 23% (3) neither agreed nor disagreed.

**DISCUSSION**

**Bulrush Response to Flooding, Salinity, and Fertilization**

We found that emergence of *S. acutus* was strongly affected by water-depth, and salinity affected *S. acutus* plant growth. In contrast, all *B. maritimus* plants emerged and neither water-depth nor salinity strongly affected growth, though salinity did affect plant height under high nutrients. These findings suggest that selection of sites with suitable environmental conditions to meet the species-specific requirements can help improve plant establishment in GSL wetland restorations.

Water-depth was a significant factor for emergence for *S. acutus*. Though *S. acutus* is a species that often occupies depths of up to 150 cm, recent studies suggest survival and establishment are poor in flooded conditions (Dabbs 1971; Tilley 2012;
Sloey et al. 2015a; Sloey et al. 2015b). The prevention of emergence could be due to a variety of factors associated with water-depth including low light availability and low-oxygen conditions. However the high water-depth treatment was just 10 cm; in studies of species closely related to *S. acutus*, light extinction data suggested that light availability should not be limited until depths of 75-90 cm (Squires and Van der Valk 1992). At shallow depths, oxygen depletion in the root zone inhibits rhizome bud growth for other *Schoenoplectus* species (Laing 1941). In the field, it is hypothesized that *S. acutus* in GSL wetlands escapes anaerobic stress by using shoots from previous growing seasons to transport oxygen (Smith and Kadlec 1985). A study examining a California salt marsh restoration reported that adult *S. acutus* with stems attached established much better than rhizome transplants, likely due to oxygen transport from stems to root systems (Sloey et al. 2015). A reliance by *S. acutus* on aboveground culms to access oxygen is consistent with our results indicating that, if *S. acutus* rhizomes emerged, water-depth did not have an effect on growth. Emergent plants have numerous adaptations to overcome oxygen depletion, including aerenchyma formation, shoot elongation, reduction of transpiration rates, formation of shorter roots and narrower steles, and induction of anaerobic metabolism (Blom et al 1994; Jackson and Armstrong 1999; Voesenek et al 2004; Kreuzwieser et al 2004; Armstrong and Beckett 1987; Vartapetian and Jackson 1997). It is likely that *S. acutus* rhizomes either remained dormant in response to the anaerobic environment or used adaptations to anaerobic conditions to overcome submergence. Timing of collection could also have complicated dormancy release, as rhizomes are more nutrient depleted in the fall (the season when the rhizomes for the present study were collected) (Chancellor 1974; Vanderbosch and Galatowitsch 2010). Further,
because culms were trimmed following collection, rhizomes did not have access to atmospheric oxygen. Therefore, when planting *S. acutus*, it is important to consider factors affecting oxygen availability. For example, if flooding is desirable or unavoidable, non-dormant plantings or plantings with aboveground stems may be appropriate, as this approach will allow the plant to use adaptations to overcome anaerobic conditions. Timing of planting and collection may also be considered to prevent use of dormant materials.

Our results further indicate that *B. maritimus* is more salinity tolerant than *S. acutus*. Salinity had a negative effect on *S. acutus* growth, while *B. maritimus* growth was not affected by salinity. However, *B. maritimus* growth did improve under low salinity combined with high nutrients. Field observations and experiments support the contention that *B. maritimus* displays greater salinity tolerance than *S. acutus* [e.g. the presence of *B. maritimus* in hypersaline lakes and its seedling survival in high salinity (Hammer and Heseltin 1988; Kadlec and Smith 1989; Downard unpublished data)]. Further, a European greenhouse experiment demonstrated that *B. maritimus* is one of the most salt tolerant plants of its genus (Hroudová et al 2014).

We were surprised to find that nutrients had a positive effect on *B. maritimus* growth, given that a previous greenhouse experiment *B. maritimus* had the lowest biomass and stem density found of three other *Bolboschoenus* species under high nutrient conditions (Hroudová et al 2014). In the absence of competition, it is intuitive that nutrients improve plant growth (Epstein 1972). Of perhaps greater significance is that salinity does not have a strong negative effect on *B. maritimus* emergence and growth. Hroudová et al. (2014) proposed that *B. maritimus* is evolutionarily adapted to out-
competing other plant species in highly stressful environments where nutrients are low and salinities are high. These results suggest that salinity management in impoundments can influence *B. maritimus* and *S. acutus* establishment and distribution. *Bolboschoenus maritimus* may be more likely to outcompete other species in high-salinity environments, and *S. acutus* may thrive in low to moderate-salinity impoundments. However, managing salinity in impounded wetlands may be difficult, as duration of inundation in addition to interactions between depth and salinity strongly impact salinity (Smith and Kadlec 1983; Watt et al. 2007; Downard unpublished data). For example, lower water-levels may help *S. acutus* establishment by alleviating anaerobic conditions, but may simultaneously increase the salinity concentration, thereby inhibiting *S. acutus* establishment.

**Trustee Involvement in Research Project and Response to Enrichment Activities**

Collaboration between the SLCJ and researchers was successful. The involvement of the SLCJ was valuable to our research through provision of labor, facilities, and ecologically coherent results. Trustees provided the majority of labor, logging 320 hours compared to 160 researcher and staff hours. Trustees provided treatment application, data collection, and basic maintenance, and their daily time spent on the project allowed them to make detailed observations and to help solve problems as they arose. The SLCJ also provided a large greenhouse space that would otherwise be unavailable to researchers. The project produced logical results, signaling that the trustees’ attention to detail and their ability to follow protocol were trustworthy. Our biological conclusions from this study are supported by prior studies on related species and by more general botanical literature (e.g. nutrients improve plant growth) (Epstein 1972; Smith and Kadlec 1989;
Hroudová 2014). For basic biological studies, given sufficient provisions for trustee training and oversight, jails are a viable venue and an excellent resource for researchers and conservationists.

Importantly, trustees responded positively to participation in our research project, despite relatively brief involvement due to short sentence lengths at the jail. The majority of the trustees we surveyed reported that they improved their knowledge of plant science, were encouraged to continue their education, and enjoyed the program. Though sample size was small, assessment tools suggest that project participation stimulated interest in education and generally provided a positive experience for trustees. Anecdotally, some trustees took ownership and control over aspects of the project by solving problems with water-monitoring wells, improving organization of data collection and sampling tools, and proposing their own hypotheses about how plants were responding to different treatments. A connection to wetland science and GSL environmental issues was evident from trustees’ descriptions of *Phragmites* patches they personally identified on jail property and their desire to transplant *B. maritimus* and *S. acutus* in wet areas of the jail property after completion of the project.

Challenges to the research project mostly had to do with short trustee sentences and relatively high trustee turnover, but overall they were not a major problem. Ensuring protocols were performed consistently required repeated re-training sessions, trustee-to-trustee training, and weekly maintenance evaluations by researchers and staff. Further research on the amount of supervision required and the effectiveness of trustee-to-trustee training could increase project efficiency in jails. High trustee turnover also made it somewhat difficult to capture all trustee feedback, as we were not always aware when
trustees would be leaving the program and unable to administer surveys to all who participated. Future studies could concentrate more explicitly on the relationship between exposure to the research project and benefits to the trustee.

Conclusions

This is the first research project within the SPP Network to be completed by inmates in a jail setting and can serve to inform future work of the SPP Network. This collaboration benefitted both researchers and the jail population by delivering therapeutic and educational value to an underserved demographic, while simultaneously addressing pressing research questions to guide GSL wetland restoration efforts. The jail partnership led to critical research findings concerning the impact of salinity and water-depth on *B. maritimus* and *S. acutus* emergence and growth. Specifically, we found that *B. maritimus* is reasonably salinity tolerant and should establish across a range of nutrient, salinity, and flooding conditions. But care in site selection, water management, and salinity management need to be considered for *S. acutus* due to the impact of water-depth on emergence of this species.

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Hroudová, Z., Z., and F. Monika. 2014. The tolerance to salinity and nutrient supply in four European Bolboschoenus species (B. maritimus, B. laticarpus, B. planiculmis and B. yagara) affects their vulnerability or expansiveness. Aquatic Botany 112:66-75.


TABLES AND FIGURES

Table 1: ANOVA results for the effect of water depth, nutrients, and salinity for *B. maritimus*.

<table>
<thead>
<tr>
<th></th>
<th>DF</th>
<th>Plant height F-value (p-value)</th>
<th>Stem density F-value (p-value)</th>
<th>Total biomass F-value (p-value)</th>
<th>Aboveground biomass F-value (p-value)</th>
<th>Belowground biomass F-value (p-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth</td>
<td>1,33</td>
<td>1.57 (0.22)</td>
<td>0.84 (0.37)</td>
<td>0.95 (0.34)</td>
<td>2.50 (0.13)</td>
<td>1.13 (0.30)</td>
</tr>
<tr>
<td>Nutrients</td>
<td>1,33</td>
<td>19.21</td>
<td>&lt;0.0001</td>
<td>9.35 (0.005)</td>
<td>3.98 (0.06)</td>
<td>7.90 (0.01)</td>
</tr>
<tr>
<td>Salinity</td>
<td>1,33</td>
<td>0.003 (0.95)</td>
<td>10.90 (0.002)</td>
<td>2.82 (0.10)</td>
<td>0.20 (0.66)</td>
<td>0.33 (0.57)</td>
</tr>
<tr>
<td>Depth*nutrients</td>
<td>1,33</td>
<td>0.00 (0.99)</td>
<td>0.05 (0.82)</td>
<td>0.03 (0.87)</td>
<td>0.00 (0.96)</td>
<td>0.09 (0.77)</td>
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<tr>
<td>Depth*salinity</td>
<td>1,33</td>
<td>0.85 (0.36)</td>
<td>0.13 (0.72)</td>
<td>0.10 (0.75)</td>
<td>0.93 (0.34)</td>
<td>2.91 (0.10)</td>
</tr>
<tr>
<td>Nutrients*salinity</td>
<td>1,33</td>
<td>1.75 (0.20)</td>
<td>4.58 (0.04)</td>
<td>3.50 (0.07)</td>
<td>1.78 (0.19)</td>
<td>3.38 (0.08)</td>
</tr>
<tr>
<td>Depth<em>nutrients</em>salinity</td>
<td>1,33</td>
<td>0.42 (0.52)</td>
<td>0.14 (0.71)</td>
<td>0.21 (0.65)</td>
<td>0.36 (0.55)</td>
<td>0.03 (0.88)</td>
</tr>
</tbody>
</table>
Figure 1: Effects of depth, nutrients, and salinity on emergence, mean peak stem density (± 1 SE), mean peak plant height (± 1 SE), and mean total biomass (± 1 SE) of *B. maritimus* and *S. acutus*. Aboveground and belowground biomass data available in supplemental results.
Figure 2: Effects of depth, nutrients, and salinity on mean aboveground biomass (± 1 SE) and mean belowground biomass (± 1 SE) of *B. maritimus* and *S. acutus*.
To regain the important ecosystem services provided by wetlands requires a detailed understanding of the biological traits of plant species vital to wetland restoration. In this thesis we reported findings that improve the knowledge of seed dormancy break, seed germination, and rhizome growth for three globally important bulrush species (*Bolboschoenus maritimus*, *Schoenoplectus acutus*, and *S. americanus*) critical to revegetation of Great Salt Lake (GSL wetlands).

### Seed dormancy break and germination of *B. maritimus*, *S. acutus*, and *S. americanus*

In Chapter Two of this thesis, we developed guidance for the seed dormancy break and germination requirements of *B. maritimus*, *S. acutus*, and *S. americanus*. For *B. maritimus*, 180 day cold, moist stratification and bleach scarification are effective dormancy break treatments. For *S. acutus* 180 day cold, moist stratification was the most effective dormancy break treatment in one experiment, but 30 day stratification improved germination comparably in two other experiments. Longer stratification lengths for *S. acutus* may result in more consistently high germination percentages, but 30 day stratification may be sufficient length for some sites. For *S. americanus*, stratification improved germination in two of three experiments, but 20-60% of viable seed did not germinate across all experiments, potentially due to a poor understanding of germination requirements. Soaking seeds improved the germination of all species when applied after dormancy break treatments. Importantly, response to dormancy break treatment varied among sites and intraspecific variation may account for unpredictable responses to
dormancy break treatments. Taken together, these findings can assist practitioners to craft strategies to effectively break dormancy and germinate seeds of *B. maritimus*, *S. acutus*, and *S. americanus*.

**Environmental factors affecting emergence and growth of *B. maritimus* and *S. acutus***

In Chapter Three of this thesis we determined how water depth, nutrient, and salinity levels affect *B. maritimus* and *S. acutus* emergence and growth from rhizomes. High water depth negatively affected emergence of *S. acutus*, likely due to decreased oxygen availability. Care in plant materials selection and hydrologic management must be taken when planning revegetation using *S. acutus* rhizomes, such as use of plant material with aboveground biomass present and drawdown of the revegetation site. Additionally, *S. maritimus* was found to be reasonably salinity tolerant and should be able to establish in a variety of water depth, nutrient, and salinity conditions.

**Conclusion**

This thesis investigated basic biological traits relating to revegetation of the globally important bulrushes *B. maritimus*, *S. acutus*, and *S. americanus* in GSL wetlands. The findings of this thesis can be used by wetland managers to improve establishment of *B. maritimus*, *S. acutus*, and *S. americanus* via seed and rhizomes.