

AN ABSTRACT OF THE DISSERTATION OF

Elena Del Giudice Tuttle for the degree of Doctor of Philosophy in Environmental Science presented on August 12, 2021.

Title: Ecotypic Variation of *Schoenoplectus pungens* (Vahl) Palla in Response to Salinity and Inundation

Abstract approved:

Dennis A. Albert

Local adaptation in plants may hold the key to understanding the level of resilience of an ecosystem and probability of persistence of a species in the face of rapid anthropogenic changes in climate and disturbance regime. Clonal species are especially important in wetlands, one of our most productive and vulnerable ecosystems. Not only have wetlands already been subject to habitat fragmentation and loss but they are expected to experience dramatic change in the next century. When dealing with fragmented populations and potentially small effective population sizes, understanding population dynamics and response to abiotic stress from a clonal species perspective will allow for informed management, restoration, and policy in their regards.

One such group of clonal plants, the sedges, occupy a wide range of habitats across all climates, with Cyperaceae being one of the largest cosmopolitan families of angiosperms, including about 90 genera and 5500 species (Leck and Schütz, 2005; Christenhusz and Byng, 2016). Sedges are often dominant species in wetlands, where they can form large monospecific stands, as is the case for the species of interest, *Schoenoplectus pungens* (Albert et al, 2013; Schütz, 2000; Leck and Schütz, 2005; Marty and Kettenring, 2017).

In chapter two, the focus was on determining which stratification techniques might improve germination success in *S. pungens* and whether stratification preference may have an ecotypic component. Seeds were collected from Big Lagoon, CA, Lake Earl, CA, Coos Bay, OR, Necanicum River, OR, Gray's Harbor, WA, and Skagit River, WA. Sites were chosen based on the availability of fertile material at the time of collection and because they were representative of the study area. Seeds were stratified using different moisture and temperature conditions, with the hypothesis that wet stratification would have higher germination than dry, and ultimately, cold and wet stratification would result in the highest germination rates. Results demonstrated that not only can germination success for certain populations can be higher than previous studies suggest, but that ideal conditions for germination may be dictated by source populations. The percent germination ranged from a low of 0% (Skagit River, WA) following warm dry stratification to a high of 76% (Big Lagoon, CA following warm dry stratification and Skagit River, WA following cold wet stratification). This study suggests that success in propagation from seed in greenhouse conditions for use in wetland restoration is entirely possible for *Schoenoplectus pungens*, but depends on the site of origin of seeds and stratification method used. The results suggest that implementation of wet and cold stratification is the most consistent method to yield higher germination if site of origin is unknown.

Seeds from the germination study sites were utilized in a common garden experiment using constructed wetland mesocosms, the results of which form the basis for both Chapter three and Chapter four. In Chapter three, the primary question of interest was whether different populations (same sites as in chapter two) of

Schoenopletus pungens showed variation in growth to salinity and inundation. A further question centered on whether changes in salinity and inundation would result in shifts in allocation between sexual and asexual reproduction. Findings of significant interactions between salinity, inundation, and source population would provide evidence for differential adaptation, while no between-site differences would support phenotypically plastic responses. The results show that salinity, inundation, and source population all impacted the proportion of fertile buds, maximum height, and the number of culms produced, although to varying extent. High salinity combined with tidal conditions not only reduced the overall number of culms, but also reduced the proportion of culms producing inflorescences and the total number of fertile buds. Our results indicated some trade-offs between sexual and asexual reproduction with life-stage, specifically between the first and second year of growth, with no fertile material being produced in the first year in favor of vegetative growth. Between site differences in response to salinity and inundation were most marked for Coos Bay, OR, Big Lagoon, CA, and Skagit River, CA. Coos Bay, OR seemed to have a lower tolerance to the high salt treatment as it generally had the lowest maximum height and live culm number. Skagit River, WA had taller culms but generally fewer of them, especially in brackish and salt treatments. The remaining sites were virtually indistinguishable in their growth response. This study shows that both local adaption and phenotypic plasticity may play a role in vegetative response to salinity and inundation, and that the degree of local adaptation may differ between source populations. This shows that understanding reproduction dynamics, even in species with perceived low sexual reproduction, is important from a conservation perspective.

Chapter four investigated the impacts of salinity and inundation on biomass production and allocation of *S. pungens*, with seeds drawn from the germination portion of the study. Results showed that under consistent inundation, *S. pungens* can withstand near seawater salinity concentrations. However, when high salinity is paired with daily drying *S. pungens* biomass production, and presumably long-term survival, suffer. The results of the biomass allocation analysis suggest that below-

ground biomass is disproportionately important to *S. pungens*. Together, Chapters three, four, and five looked at the possibility of ecotypic variation in the response variables of germination, sexual and asexual allocation, general morphology (maximum height and culm number), and above-and below-ground biomass allocation. The research has shown that there is some ecotypic variation of *Schoenoplectus pungens* during germination and growth, indicative of local adaptation, but that the signal differs with source populations.

S. pungens is of ethnobotanical importance in the upper Pacific Northwest portion of its coastal range. Although *S. pungens* is not currently at risk of extinction, ranges in tribal areas have experienced decline in extent and quality (personal communication, Snowhomish Tribe). Chapter five provides a link between western science (eg. Chapters 3-5), traditional ecological knowledge, and conceptualization of the risk of species loss. This chapter hopes to illustrate how this connubium can improve extinction risk and uncertainty quantification in conservation biology and endangered species legislation by including concepts such as TEK (traditional ecological knowledge) and CKS (cultural keystone species). The proposed framework of risk assessment, conceptualized for salmonids, seeks to apply coupled human-natural systems science approach to improve TEK and CKS assessment and protection. This holistic approach to endangered species conservation prioritization could result in more informed actions and funding decisions that align societal and cultural concerns with environmental and ecological ones. Not only will this approach give more ‘power’ to protective legislation, it will enfranchise and engage a larger portion of the population - particularly disenfranchised communities. Hopefully, studies such as the foregoing chapters can aid tribal biologists and tribal elders in assessing risk to important harvest stands of *S. pungens* and inform managed and restoration efforts going forward.

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Ecotypic Variation of *Schoenoplectus pungens* (Vahl) Palla in Response to Salinity
and Inundation

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I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Elena Del Giudice Tuttle, Author

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CONTRIBUTION OF AUTHORS

Chapter 4 was written in partnership with Patricia Halleran, a doctoral student in the department of Anthropology. This chapter is in fulfillment of the minor in “Risk and Uncertainty Quantification in Marine Science”, a National Science Foundation Research Traineeship.

TABLE OF CONTENTS

	<u>Page</u>
1 - General Introduction.....	1
2 - Ecotypic variation of germination success of <i>Schoenoplectus pungens</i> (Vahl) Palla following stratification utilizing varying temperature and moisture	3
2.1 Introduction	4
2.2 Methods	8
2.3.1 Statistical Methods	10
2.4 Results	11
2.5 Discussion:	15
2.6 Conclusion.....	18
3 - Ecotypic variation in growth and reproductive plasticity of <i>Schoenoplectus pungens</i> (Vahl) Palla under varying inundation and salinity treatments	20
3.1 Introduction	21
3.2 Methods:.....	25
3.2.1 Statistical Methods:	26
3.3 Results:	26
3.3.1 Proportion of fertile culms:.....	26
3.3.2 Live culms per pot:	30
3.3.3 Maximum height.....	33
3.4 Discussion:	37
3.5 Conclusion:.....	39
4 - Effects of salinity and inundation on biomass production and allocation in an obligate wetland monocot, <i>Schoenoplectus pungens</i> (Vahl) Palla	40
4.1 Introduction	41
4.2 Methods.....	46
4.2.1 Field Sampling:.....	46
4.2.2 Common Garden Methods:	48
4.2.3 Statistical Methods	50
4.3 Results	52

4.3.1 Above-ground biomass.....	56
4.2.2 Below-ground biomass results.....	58
4.2.3 Total biomass results	59
4.2.4 RSR results	61
4.4 Discussion	63
4.4.1 Biomass production	63
4.4.1 Site of origin	67
4.4.3 Biomass allocation.....	67
4.5 Conclusion.....	69
5 - Assessing TEK: Applying Ecological Frameworks to Human and Cultural Keystone Species Relationships.....	71
5.1 Introduction	72
5.2 Background	73
5.2.1 Traditional Ecological Knowledge (TEK) and Cultural Keystone Species	73
5.2.2 Cultural Keystone Species and Human Rights.....	82
5.3 Extinction	85
5.3.1 Current Legislation	86
5.4 Framework development.....	91
5.4.1 Existing frameworks and creating a shared lexicon	92
5.4.2 Methods	93
5.5 Discussion	96
5.6 Conclusion.....	100
5.7 References	102
6 – Summary and Conclusion	114
7 - Bibliography	116

LIST OF FIGURES

<u>Figure</u>	<u>Page</u>
Figure 2.1: A: close up of Three Square Bulrush. B: Proposed Pacific Coast sub-species (var. <i>badius</i>) range in North America. C: drawing description of Bulrush.....	4
Figure 2.2: a) ten <i>S. pungens</i> achenes placed on Whatman filter paper moistened with DI water b) sealed petri dish containing <i>S. pungens</i> achenes c) germination set-up with timed artificial light	10
Figure 2.3: Estimated mean percent germination by site of origin and stratification method ($\pm$ 95% confidence interval) of <i>S. pungens</i> collected along the Pacific Coast of North America. ...	12
Figure 3.1: Map showing study sites of <i>S. pungens</i>	25
Figure 3.2: Graphs of the estimated marginal means ($\pm$ 95% CI) of the proportion of fertile culms per pot by <i>Schoenoplectus pungens</i> individuals from six geographically distinct source populations under varying salinity treatments and inundation conditions.....	27
Figure 3.3: The estimated marginal means ($\pm$ 95% CI) of live culms produced per pot by <i>Schoenoplectus pungens</i> individuals from six geographically distinct source populations under varying salinity treatments and inundation conditions.	30
Figure 3.4: The estimated marginal means ($\pm$ 95% CI) of the maximum culm height per pot by <i>Schoenoplectus pungens</i> individuals from six geographically distinct source populations under varying salinity treatments and inundation conditions.	33
Figure 4.1: Sketch of <i>Schoenoplectus pungens</i> showing fertile culms and turf-like fibrous roots and thick rhizomes. (By Mary Barnes Pomeroy. Copyright © 2020 Estate of Herbert Mason.)	46
Figure 4.2: Estimated marginal means $\pm$ 95 % CI of above-ground biomass under different salinity and inundation treatments grouped by site of origin.....	56
Figure 4.3: Estimated marginal means $\pm$ 95 % CI of below-ground biomass under different salinity and inundation treatments grouped by site of origin.....	58
Figure 4.4: Estimated marginal means $\pm$ 95 % CI of total biomass under different salinity and inundation treatments grouped by site of origin.	60
.....	62
Figure 4.5: Estimated marginal means $\pm$ 95 % CI of RSR under different salinity and inundation treatments.....	62
Figure 5.1: Handbook for Culturally responsible Science Curriculum. Stephens, Sidney 2000..	75

LIST OF TABLES

<u>Table</u>	<u>Page</u>
Table 2.1: Estimated marginal means of percent germination by site and stratification method of <i>S. pungens</i> collected along the Pacific Coast of North America.	13
Table 2.2: Pairwise differences of estimated marginal means with Bonferroni adjustment within sites between stratification methods of <i>S. pungens</i> collected along the Pacific Coast of North America. Significant differences appear in bold with asterisks.....	14
Table 2.3: Excerpt of significant pairwise differences of estimated marginal means with Bonferroni adjustment within stratification methods between sites of <i>S. pungens</i> collected along the Pacific Coast of North America. Significant differences appear with asterisks.	15
Table 3.1: Summary comparisons in estimated marginal means of the proportion of fertile culms per pot by <i>Schoenoplectus pungens</i> individuals from six geographically distinct source populations under varying salinity treatments and inundation conditions. All comparisons were conducted using all grouping variables.If a grouping variable was not found to differ in estimate, it was collapsed for this table. See Appendix 1 for numerical values of the comparison results. 28	
Table 3.2: Simplified comparisons in estimated marginal means of live culms produced per pot by <i>Schoenoplectus pungens</i> individuals from six geographically distinct source populations under varying salinity treatments and inundation conditions. All comparisons were conducted using all grouping variables. If a grouping variable was not found to influence the comparison, it was collapsed for this table. See Appendix 1 for numerical values of the comparison results	31
Table 3.3: Simplified statistical comparisons of estimated marginal means of live culms produced per pot by <i>Schoenoplectus pungens</i> individuals from six geographically distinct source populations under varying salinity treatments and inundation conditions. All comparisons were conducted using all grouping variables.If a grouping variable was not found to influence the comparison, it was collapsed for this table. See Appendix 1 for numerical values of the comparison results.	34
Table 4.1: GLM model terms.....	52
Table 4.2: For all response variables (above, below, total, and root-to-shoot ratio) the model with lowest AIC was selected, resulting in the following models:.....	53
Table 1: Characteristics of an ideal classification of threats (Salafsky, 2007)	94
Table 5.2: Threat impact generated by considering scope and severity of major threats	96
Table 5.3: Conceptual Framework Summary	99
Table 5.4: Contributing factors (amplification)	100

DEDICATION

Dedicated to my grandparents, without whom I would not have been able to pursue my studies. Thank you.

1 - GENERAL INTRODUCTION

Impacts to coastal wetlands have been both numerous and variable in their results. Today, remaining coastal salt marshes are recognized for their importance both ecologically and from an ecosystem services perspective. Studies have shown primary production values to be among the highest (Montague and Wiegert 1990; Odum and McIvor 1990) and they are important in sediment stabilization, wildlife habitat, aesthetic values, cultural importance, and storm protection (Kennish, 2002; Zedler, 2001; Turner, 2004; Taylor, 2012). Beginning with extensive environmental legislation in the 1970s, a two pronged approach of wetland conservation and restoration has been implemented. As existing coastal wetlands are protected, attention is being given to the condition of existing wetlands and restoration actions to improve or expand wetland habitat. More recently, it has become of mounting importance and urgency to understand the effects of climate change, sea level rise, and the possibly rapid and catastrophic impacts these changes might have on highly delicate and complex coastal systems. Understanding both the risk and uncertainty associated with the persistence of both biotic and abiotic systems and the human and animal populations that rely upon them, has become paramount. The research here presented hopes to begin to unravel the possible effects that changing salinity and inundation regimes may have on *Schoenoplectus pungens*. At a finer scale, this study is interested in possible ecotypic differences in growth response to these abiotic stressors (Chapters three and four) and in the successful germination under varying stratification conditions (Chapter two). The seeds for this series of experiments were harvested following two years of field surveys along the Pacific Coast. The primary interest was populations in contact with high salinity during a portion of the tidal cycle and fertile populations with these conditions only occurred beginning in Northern California. It was suspected that this was due to increasing salinity in more southern sites, but this will require further investigation. Therefore, seeds were taken from six Pacific Coast populations ranging from northern California through Oregon and Washington, with two sites in each state at fairly regular distances: Big Lagoon, CA, Lake Earl, CA, Coos Bay, OR, Necanicum River, OR, Gray's Harbor, WA, and Skagit River, WA. Chapters three through five trace the same individuals from germination to growth in wetland mesocosms to biomass harvest at the end of two growing seasons.

Chapter two hopes to determine germination success in *S. pungens* to improve our understanding of seed dynamics and determine if there is a genetic basis for germination variability. We test variable moisture and temperature conditions with the hypothesis that wet stratification would have higher germination than dry, and ultimately, cold and wet stratification would result in the highest germination rates.

Chapter three asks if *S. pungens* from different source populations shows variation in growth response to salinity and inundation, focusing on maximum culm height and mean live culms. A corollary question centers on the reproductive response of *S. pungens* to changes in salinity and inundation indicated by changes in allocation between sexual and asexual reproduction.

Chapter four expands upon the previous chapter by investigating the impacts of salinity and inundation on biomass production and allocation after two growing seasons. Response variables include above-, below-, and total biomass as well as a calculated root-to-shoot ratio. Biomass allocation is important in wetland species as it can help with modeling and forecasting rates of carbon sequestration, nutrient cycling, and shoreline stability (as well as other ecosystem services).

Chapter five was the result of a transdisciplinary collaboration with an anthropologist as part of the National Science Foundation Research Traineeship. It proposes a novel framework for risk assessment in the context of species and biodiversity loss by applying coupled human-natural systems science approach to existing frameworks by incorporating improved TEK and CKS assessment and protection. Although the framework presented focuses on salmonids as the model organism, it is applicable to a wide variety of species. *S. pungens*, although not currently endangered, is a species of ethnobotanical importance in portions of its range and as such could benefit from risk assessments that includes both a scientific and TEK approach. Knowledge exchange between stakeholders will benefit future risk assessment and management decisions in the context of changing land use, climate change, and sea level rise.

**2 - ECOTYPIC VARIATION OF GERMINATION SUCCESS OF
SCHOENOPLECTUS PUNGENS (VAHL) PALLA FOLLOWING
STRATIFICATION UTILIZING VARYING TEMPERATURE AND MOISTURE**

2.1 INTRODUCTION

Sedges occupy a wide range of habitats across all climates, with Cyperaceae being one of the largest cosmopolitan families of angiosperms, including about 90 genera and 5500 species (Leck and Schütz, 2005; Christenhusz and Byng, 2016). Sedges are often dominant species in wetlands, where they can form large monospecific stands as is the case for *S. pungens* (Albert et al, 2013; Schütz, 2000; Leck and Schütz, 2005; Marty and Kettenring, 2017). Reproductive strategies in sedges are highly variable, with some annual species reproducing only from seed while other species produce few seeds, thus relying on vegetative spread (Leck and Schütz, 2005). Of those species that produce seed, viable seed production can be highly variable between years, between populations, or even between stands within the same population (Leck and Schütz, 2005). In highly fragmented habitats, re-establishment of sedge-dominated communities without human intervention may be limited and propagation from seed could be an economically and spatially efficient revegetation strategy compared with plugs or rhizome propagation (Leck and Schütz, 2005; Palmerlee and Young, 2010). Germination in sedges is often difficult due to seed dormancy and hard seed coats found in many species (Baskin and Baskin, 2004). Studies on seed dormancy break and germination rates are limited for many important habitat-forming species (Marty and Kettenring, 2017), especially those that appear to

reproduce predominantly vegetatively.

Schoenoplectus pungens (common three-square bulrush)

is a wetland species found throughout North America in inland and coastal

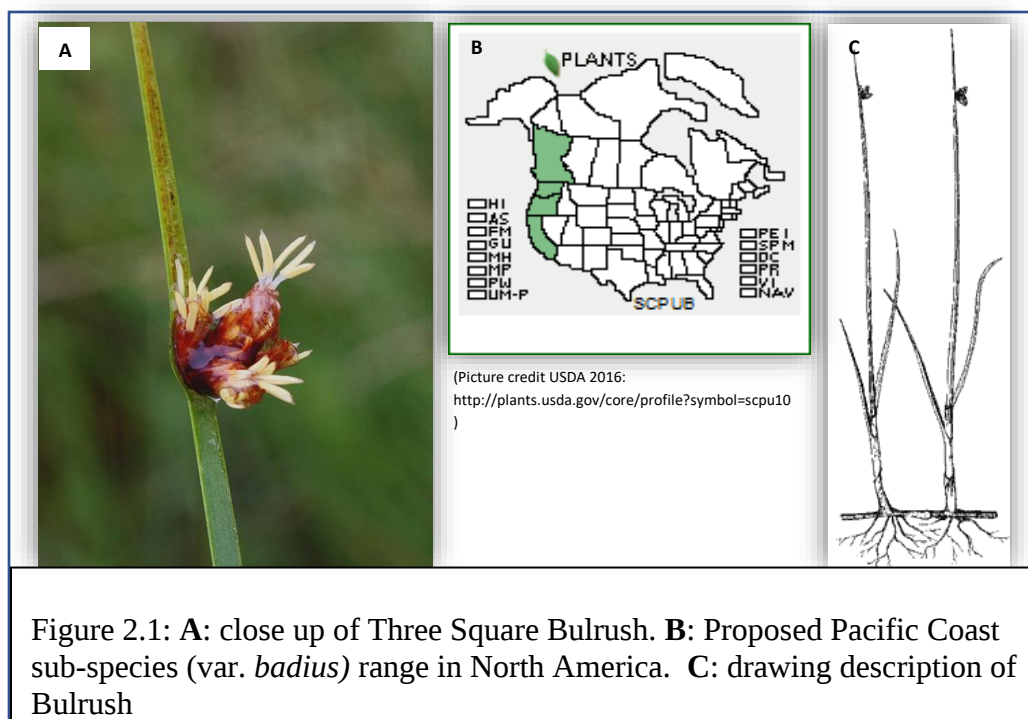


Figure 2.1: **A:** close up of Three Square Bulrush. **B:** Proposed Pacific Coast sub-species (var. *badius*) range in North America. **C:** drawing description of Bulrush

freshwater and brackish wetlands, occupying the low and mid marsh zone where it attenuates wave energy and stabilizes shorelines (Albert, et al. 2013, Yoon et al. 2011). Figure 2.1 shows the proposed distribution of *S. pungens* var. *badius* on the Pacific Coast, although it is not yet recognized. This plant is used in wetland restoration for shoreline stabilization, habitat creation, and reestablishment of culturally important harvest beds for basketry and other ethnobotanical uses (Albert et al. 2013, Stevens et al. 2012, Harwell, 2015; Crandell, 2018). Restoration of bulrush beds is often achieved by transplanting turf slabs or rhizome plugs from existing marshes (Stevens et al. 2012, Harwell, 2015, Crandell, 2018). This method requires large extant marshes and often results in transplanting material from geographically or genetically different populations. Planting from seed has been regarded as inefficient due to low germination success and low recruitment (Neff et al. 2005; Thomsen et al. 2005). Sexually produced seedlings have been found to have later emergence and reach lower heights compared to shoots produced by rhizomes (25cm versus 45-90cm respectively) (Giroux and Bedard, 1988).

Very little is known regarding *S. pungens* type of seed dormancy or dormancy break mechanisms and is limited to a single study more than 70 years ago and with limited replication from a single source population from the East Coast (Isley, 1944). Although capable of sexual reproduction, *S. pungens* seedlings have rarely been found in the field (Giroux and Bedard, 1988; Albert et al. 2013) and therefore this species is assumed to reproduce predominantly through rhizomes. In a study of populations of *S. pungens* along the St Lawrence River sexual reproduction represented less than 1% of new shoots compared to those produced vegetatively from rhizomes (Giroux and Bedard, 1988). Similarly, low seedling numbers were reported for Tillamook Bay, Oregon and Cecil Bay, Michigan (Albert et al., 2013). Studies of *Typha* and other clonal plants suggest that sexual recruitment is rare in mature stands, but that sexual reproduction may be the primary strategy for long-distance dispersal or re-emergence following long periods of dormancy during unfavorable conditions (Clevering, 1995; Eriksson, 1992; McNaughton, 1975; Grace and Wetzel, 1981; Gopal and Sharma, 1983). Dispersal mechanisms have been observed for many clonal emergent macrophytes and include wind, water, or animals (Cook, 1987; Baskin and Baskin, 2014; Eriksson, 1992; Waisel, 1972). *S. pungens* has a high potential for dispersal by a variety of abiotic and biotic vectors as seen in closely related species, especially through waterfowl, as *S. pungens* is an important forage species for several species

along the Pacific and Great Lakes Flyways (Giroux et al. 1995). Spatial separation between seedlings and existing stands may be an additional factor contributing to the lack of observations of seedlings in the field.

Notwithstanding few observations of seedling in the field, reproduction from seed may play an important role in dispersal, persistence, and gene flow of *S. pungens*, as it does in *Carices* (Schütz, 2000). A comprehensive review of Cyperaceae seed ecology and dynamics suggests that seed bank persistence and germination is important for close relatives of *S. pungens* (Clevering, 1995; Schütz, 2000, Leck and Schütz, 2005), although recent direct studies of *S. pungens* do not exist. Low-risk germination strategies found in closely related species exploit temporally and spatially infrequent gaps (Leck and Schütz, 2005), therefore observations would need to coincide with these occurrences to find evidence of seedling establishment. Furthermore, findings from a review of 32 *Carex* species found that temperature induced dormancy break suggests a strict spring and summer germination period, therefore the possibility of observing seedlings in the field would be restricted to a relatively short time period (Schütz 1995). *S. pungens* grown from seed produced upwards of 20 stems within only two months, thereby making the identification of those plants grown from seed difficult later in the season, although rhizome diameter may aid in identification (Tuttle, E. unpublished data). All these factors simultaneously explain the lack of observation of seedlings in the field and the possibility that seed dynamics are more important to *S. pungens* population ecology than previously assumed. The highly stochastic nature of gap formation and specificity of favorable conditions for germination could have led to the evolution of highly selective dormancy-break mechanisms and suggest the possible formation of persistent seed banks in *S. pungens*. If this is the case, poor germination in laboratory or nursery settings may not be due to low viability but to poor understanding of dormancy-break conditions. Likewise, low success of revegetation from seed in the field may be due to recruitment occurring in different conditions than those where mature stands are found. In other words, conditions where a mature stand could thrive may not be the conditions in which seed germination occurs.

Breaking of dormancy and germination are two separate processes that are often amalgamated in germination studies focused on restoration or applied uses (Baskin and Baskin,

2004, 2014). Although this paper seeks to help improve germination for restoration efforts, it may also serve to expand our understanding of *S. pungens* seed physiology.

The specific type of seed dormancy (physiological, morphological, morphophysiological, physical and combinational) as proposed by Baskin and Baskin (2004 and 2014) is not known for *S. pungens*, although some insight may be drawn from closely related species. Here we use the definition of dormancy as a lack of germination notwithstanding favorable environmental conditions (Baskin and Baskin, 2014). Schütz (2000) characterizes *Carices* as having a combination of strict or conditional primary dormancy with a light requirement for germination and an induction of secondary dormancy in late spring in response to temperature. The morphological features of Cyperaceae seeds, including hard seedcoats, paired with low rates of germination of fresh viable seeds suggests that some form of morphological dormancy is likewise present in *S. pungens*. No studies have yet looked at hormonal effects through treatment with GA, but the importance of light (Schütz, 2000, Leck and Schütz, 2005, Tilley, 2012, Kettenring 2016) in other species suggests a hormonal basis for dormancy break.

The phenology of *S. pungens* has not been studied in detail. *S. pungens* seeds are presumed to mature at the end of the growing season, when the seeds dehisce or when the plant senescence, which may vary depending on environmental factors such as salinity or plant age (forthcoming). *S. pungens* viable seed production has been observed to be highly variable between populations and may vary with abiotic factors, including water level, temperature, salinity, and inundation, or biotic factors that include inbreeding, low population fitness, rhizome biomass, and leaf production. Dispersal mechanisms are unknown for *S. pungens* but most likely follow those seen in many other Cyperaceae, including water and biotic interactions. Dispersal by fauna most likely occurs within the same season as seed maturation but may occur following exposure in later seasons. Likewise, abiotic dispersal may occur at anytime following seed fall. The coastal populations of *S. pungens* occur in highly dynamic environments in which erosion, burial, and high water flux may move seeds within one season or expose and move existing seeds in subsequent seasons. Long term viability of *S. pungens* is unknown, but presumably follows that of close relatives with similar seed morphology (see Schütz and Rave, 1999, Schütz 1997, Schütz 2000). The timing of germination of *S. pungens* has not been studied but is assumed to be in spring or early summer, but may be later than previously assumed based

on temperature and light responses seen in other species. Improved germination of closely related species with light and high temperature combined with observations (forthcoming) that *S. pungens* seedlings do not produce seed the first year and are highly sensitive to burial, could suggest that *S. pungens* may germinate later in summer to take advantage of fewer burial events and habitat gaps created in fall and winter.

Information regarding environmental factors during the crucial stage before and during germination is lacking for *S. pungens*, as seedlings have rarely been observed (Giroux 1988; Albert et al., 2013). Likewise, specific information regarding environmental factors impacting high seed production does not exist, although observations suggest that factors are highly population dependent. A forthcoming study found that most populations sampled did not produce seed under high salt conditions, but that in certain populations more seed was produced under brackish conditions than in fresh, although all populations produced more seed in fresh water than in salt (Tuttle, E., unpublished data). Favorable salinity regimes, in addition to protecting from direct toxicity and physiological effects of salt, may signal favorable hydrological conditions, i.e. low tidal prism helps avoid burial. Due to the importance of *S. pungens* in habitat formation, many restoration efforts have sought to re-establish stands directly from seed, but have been largely unsuccessful (Thomsen et al. 2005; Neff et al. 2005; Harwell, 2015). More detailed investigation into the environmental parameters in the period following reseedling may shed light on factors which *S. pungens* finds unfavorable. The relatively low germination rates of *S. pungens* in restoration applications may be due to the difficult conditions often present early in restoration trajectories.

This study hopes to find ways of improving germination success in *S. pungens* to improve our understanding of seed dynamics and seek to understand if there is a genetic basis for germination variability. We test variable moisture and temperature conditions with the hypothesis that wet stratification would have higher germination than dry, and ultimately, cold and wet stratification would result in the highest germination rates. The question we hope to answer are 1) does stratification method influence germination rate? And 2) does site of origin influence germination rate under differing stratification methods?

2.2 METHODS

Seeds were collected in late summer from 6 different sites: 1) Big Lagoon, CA, 2) Lake Earl, CA, 3) Coos Bay, OR, 4) Necanicum River, OR, 5) Gray's Harbor WA, and 6) Skagit River, WA. The field sites in question are heavily utilized recreationally and commercially due to the habitat they provide for game and fish species. Each location had between 3 and 12 seed collection sites depending on the area of the *S. pungens* stand. Inflorescences were clipped from stems and allowed to dry for several weeks after which achenes (henceforth referred to as seeds for ease) were loosened from the inflorescence and separated from the chaff. Seeds were visually inspected and those that were damaged or shriveled were discarded. The number of seeds which were outwardly whole and healthy varied widely between sites, therefore resulting in an unbalanced design which was addressed in the statistical analysis methods.

Undamaged seeds were cleaned in a 1% bleach solution for 1 minute to kill fungus and pathogens, but the short duration probably does not qualify as scarification of the seed coat. Seeds were subsequently divided between three stratification methods: cold wet, cold dry, and warm dry (room temperature). Warm wet was not used as mold and decay was almost immediately a problem and resulted in loss of the samples. All stratification lasted approximately 5 months from the end of September to beginning of March. Cold stratification consisted of placing seed lots in a cold storage room set at 4C in the horticulture department at Oregon State University. Cold and wet stratification consisted of placing seeds in sealed petri-dish containers filled with DI water and sealed with parafilm before placing them in cold storage. Cold and dry stratification consisted of place seeds in paper bags before placing them in cold storage. Warm dry stratification consisted of placing seeds in paper bags at ambient room temperature, approximately 68-72° F in the lab.

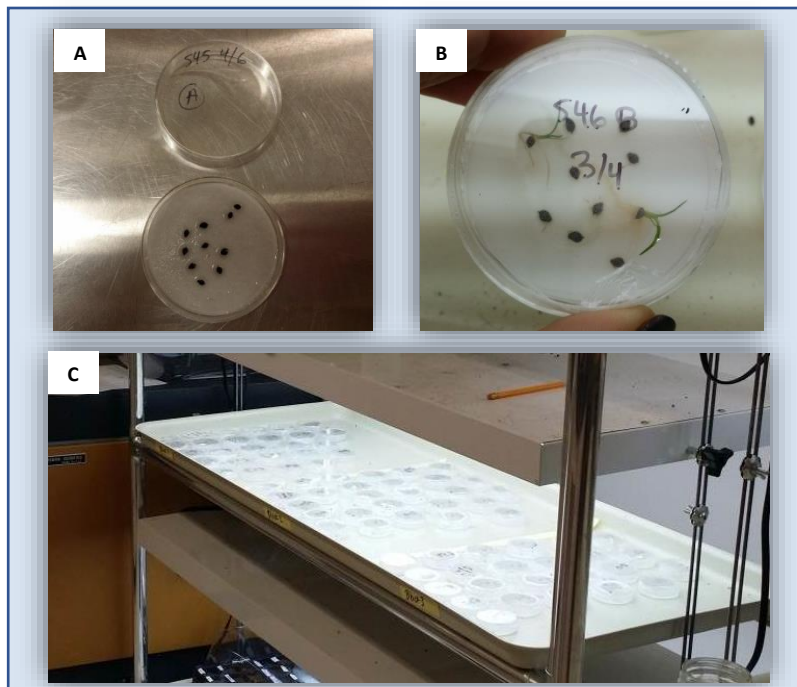


Figure 2.2: a) ten *S. pungens* achenes placed on Whatman filter paper moistened with DI water b) sealed petri dish containing *S. pungens* achenes c) germination set-up with timed artificial light

Following stratification, seeds from each collection site and stratification method were once again assessed for outward appearance of damage before being divided randomly into lots of 10 and placed in clear plastic petri dishes with nontoxic 70mm Whatman filter paper (Figure 2.2). Petri dishes were sealed with parafilm to keep out external pathogens and moistened with DI water. Petri dishes were placed randomly in 3 blocks to account for any variation in

light and temperature conditions in the germination trays. Each stratification x site combination had a minimum of 30 seeds. Temperature was maintained at 68F and HID grow lights were set to 14 hours of light per day. Total germinated seeds and non-germinated seeds were recorded every seven days beginning after the second week.

2.3.1 Statistical Methods

Percent germination was calculated from the final count data. Due to the variability in seed number from source populations and loss of seeds to fungal damage following stratification, data were unbalanced, as well as non-normal, heteroskedastic and skewed (high zeros). To account for these properties, a GLM was fitted using a quasipoisson error structure with a log link function (as a poisson model was found to be over-dispersed) (Carvalho et al., 2018). Generalized linear models (GLMs) were used to evaluate the effect of the three stratification treatments (cold wet, cold dry, and warm dry) on percent germination of seeds from the six different source populations using R (R Core Team, 2018). GLM was used to look at possible

interaction between the factors of stratification and source population. ANOVA was used to evaluate inclusion of model terms as well as comparison of the null deviance versus residual deviance. Model assumptions of normality of residuals were verified by plotting residuals versus fitted values. Estimated marginal means and subsequent pairwise comparisons between model factors with Bonferroni adjustment were attained using the package ‘emmeans’ (Lenth, 2021) in software R. Visualization of results was conducted using the package ‘ggplot2’ (Wickham, 2016). All comparisons were conducted using the emmeans package in R software and were computed using the estimate marginal means of the percent germination, thereby accounting for the unbalanced data. Results and discussion are based on estimated marginal means calculated based on the reference grids produced using the GLM.

2.4 RESULTS

Model selection procedures found the interaction term of stratification by site of origin to reduce residual deviance compared to the null model (Null deviance = 6,407.5 of 175 df, Residual deviance = 2,206.7 or 158 df). Plots of residuals to fitted values showed no significant deviation from normality.

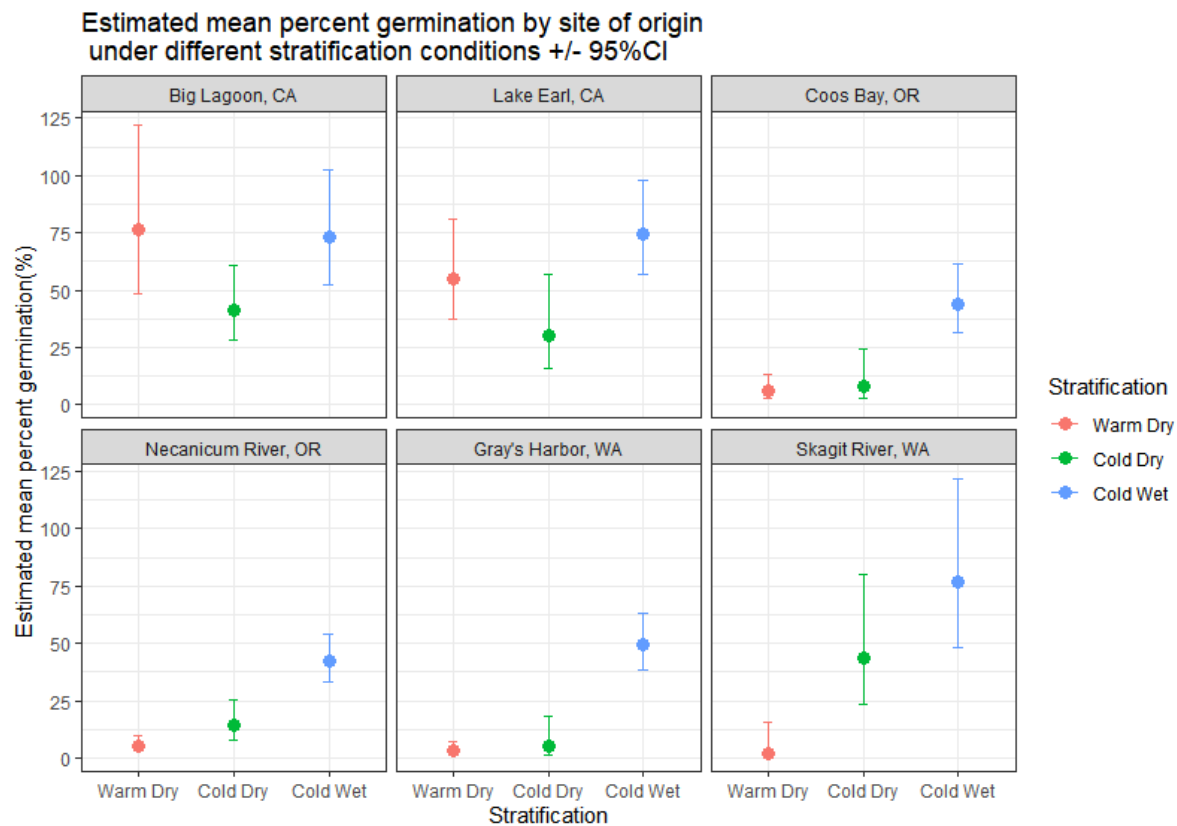


Figure 2.3: Estimated mean percent germination by site of origin and stratification method ($\pm$ 95% confidence interval) of *S. pungens* collected along the Pacific Coast of North America.

Within site comparisons showed that stratification method did effect estimated mean percent germination but that this effect differed between sites (Table 2.2 and Figure 2.3). Estimated mean percent germination following warm dry stratification for Coos Bay, OR, Necanicum River, OR, Gray's Harbor, WA, and Skagit River, WA was consistent an order of magnitude lower than mean percent germination following cold and wet stratification ($p < 0.05$ for all comparisons) (Table 2.1). Mean percent germination was higher following cold wet stratification compared to cold dry stratification for seeds from Lake Earl, CA ($44.44 \% \pm 14.25$ SE higher, $p = 0.005$), Coos Bay, OR ($36.00 \% \pm 8.79$ SE higher, $p = 0.0001$), Necanicum River, OR ($28.11 \% \pm 6.82$ SE higher, $p = 0.0001$), and Gray's Harbor, WA ($44.37\% \pm 7.10$ SE higher, $p < 0.001$). No within site difference was found in the estimated mean percent germination between warm and cold dry stratification for seeds from all sites save Skagit River, WA ($p > 0.05$ for warm dry : cold dry comparisons). For seeds from Skagit River, WA, no difference was found between the cold wet (76.67 ± 36.82 95% CI) and cold dry stratification (43.33 ± 28.44

95% CI, $p > 0.05$) but both cold stratification methods were found to have higher mean percent germination than warm dry (1.67 ± 7.6 95% CI, $p > 0.05$ for both comparisons). No statistical difference was found between stratification methods for seeds from Big Lagoon, CA ($p > 0.05$ for three comparisons).

Table 2.1: Estimated marginal means of percent germination by site and stratification method of *S. pungens* collected along the Pacific Coast of North America.

Site	Stratification	Estimated % Germination	SE	95% CI		seeds (n)	
Big Lagoon, CA	Cold Dry	41.25	8.15	28.01	60.74	16.37	80
	Cold Wet	73.33	12.54	52.45	102.53	25.04	60
	Warm Dry	76.67	18.13	48.23	121.88	36.83	30
Lake Earl, CA	Cold Dry	30.00	9.82	15.79	57.00	20.60	40
	Cold Wet	74.44	10.32	56.74	97.68	20.47	90
	Warm Dry	55.00	10.86	37.35	80.99	21.82	60
Coos Bay, OR	Cold Dry	8.00	4.54	2.63	24.31	10.84	50
	Cold Wet	44.00	7.52	31.47	61.52	15.02	100
	Warm Dry	5.83	2.50	2.52	13.52	5.50	120
Necanicum River, OR	Cold Dry	14.00	4.24	7.73	25.36	8.82	100
	Cold Wet	42.11	5.34	32.84	53.99	10.57	190
	Warm Dry	5.38	1.63	2.97	9.75	3.39	260
Gray's Harbor, WA	Cold Dry	5.00	3.27	1.39	18.04	8.33	60
	Cold Wet	49.38	6.30	38.45	63.41	12.48	160
	Warm Dry	3.33	1.34	1.52	7.32	2.90	240
Skagit River, WA	Cold Dry	43.33	13.63	23.39	80.28	28.44	30
	Cold Wet	76.67	18.13	48.23	121.88	36.83	30
	Warm Dry	1.67	1.89	0.18	15.39	7.60	60

Table 2.2: Pairwise differences of estimated marginal means with Bonferroni adjustment within sites between stratification methods of *S. pungens* collected along the Pacific Coast of North America. Significant differences appear in bold with asterisks.

Site	Contrast	difference estimate	SE	95% CI		z.ratio	p.value
Big Lagoon, CA	Cold Dry - Cold Wet	-32.08	14.95	-67.88	3.71	-2.15	0.095719
	Cold Dry - Warm Dry	-35.42	19.88	-83.01	12.17	-1.78	0.22441
	Cold Wet - Warm Dry	-3.33	22.05	-56.11	49.45	-0.15	1
Lake Earl, CA	Cold Dry - Cold Wet	-44.44	14.25	-78.55	-10.34	-3.12	0.005426 *
	Cold Dry - Warm Dry	-25.00	14.64	-60.06	10.06	-1.71	0.263348
	Cold Wet - Warm Dry	19.44	14.98	-16.41	55.30	1.30	0.582736
Coos Bay, OR	Cold Dry - Cold Wet	-36.00	8.79	-57.03	-14.97	-4.10	0.000125 *
	Cold Dry - Warm Dry	2.17	5.18	-10.24	14.57	0.42	1
	Cold Wet - Warm Dry	38.17	7.93	19.19	57.15	4.81	4.45E-06 *
Necanicum River, OR	Cold Dry - Cold Wet	-28.11	6.82	-44.43	-11.78	-4.12	0.000113 *
	Cold Dry - Warm Dry	8.62	4.55	-2.27	19.50	1.89	0.174424
	Cold Wet - Warm Dry	36.72	5.58	23.35	50.09	6.58	1.45E-10 *
Gray's Harbor, WA	Cold Dry - Cold Wet	-44.37	7.10	-61.37	-27.38	-6.25	1.24E-09 *
	Cold Dry - Warm Dry	1.67	3.54	-6.80	10.13	0.47	1
	Cold Wet - Warm Dry	46.04	6.44	30.62	61.46	7.15	2.65E-12 *
Skagit River, WA	Cold Dry - Cold Wet	-33.33	22.69	-87.64	20.98	-1.47	0.425227
	Cold Dry - Warm Dry	41.67	13.76	8.72	74.62	3.03	0.007399 *
	Cold Wet - Warm Dry	75.00	18.23	31.35	118.65	4.11	0.000117 *

Table 2.3: Excerpt of significant pairwise differences of estimated marginal means with Bonferroni adjustment within stratification methods between sites of *S. pungens* collected along the Pacific Coast of North America. Significant differences appear with asterisks.

Stratification	contrast	estimate	SE	95% CI		z.ratio	p.value	
Cold Dry	Big Lagoon, CA - Coos Bay, OR	33.25	9.32	5.88	60.62	3.57	0.005432	*
	Big Lagoon, CA - Necanicum River, OR	27.25	9.18	0.29	54.21	2.97	0.045115	*
	Big Lagoon, CA - Gray's Harbor, WA	36.25	8.78	10.48	62.02	4.13	0.000545	*
Warm Dry	Big Lagoon, CA - Coos Bay, OR	70.83	18.30	17.11	124.56	3.87	0.001635	*
	Big Lagoon, CA - Necanicum River, OR	71.28	18.21	17.84	124.72	3.92	0.001355	*
	Big Lagoon, CA - Gray's Harbor, WA	73.33	18.18	19.96	126.70	4.03	0.000825	*
	Big Lagoon, CA - Skagit River, WA	75.00	18.23	21.49	128.51	4.11	0.000584	*
	Lake Earl, CA - Coos Bay, OR	49.17	11.14	16.46	81.88	4.41	0.000154	*
	Lake Earl, CA - Necanicum River, OR	49.62	10.98	17.38	81.85	4.52	9.37E-05	*
	Lake Earl, CA - Gray's Harbor, WA	51.67	10.94	19.55	83.78	4.72	3.51E-05	*
	Lake Earl, CA - Skagit River, WA	53.33	11.02	20.98	85.69	4.84	1.97E-05	*

Within stratification treatment comparisons showed that the site of origin of seeds affected the response to stratification (Table 2.3). Seeds from Lake Earl, CA and Big Lagoon, CA had significantly higher estimated mean percent germination following warm dry stratification than all other sites by roughly 50% for Lake Earl, CA and 70% for Big Lagoon, CA (See table 2.3, $p < 0.01$ for all relevant comparisons). Following cold dry stratification, seeds from Big Lagoon, CA had roughly 30% more mean percent germination than Coos Bay, OR, Necanicum River, OR, and Gray's Harbor, WA (See table 3, $p < 0.01$ for all relevant comparisons).

2.5 DISCUSSION:

Results show that stratification method had a significant effect on estimated mean percent germination but that this effect differed between sites. The between site variability of percent germination and stratification response suggests that germination patterns in *S. pungens* have a genetic basis, indicating local adaptation. Maximum percent germination of *S. pungens* from this study was fairly high when compared to results from studies of its closest relative, *S. americanus*. Studies conducted on *S. americanus* found the highest percent germination

following cold wet germination: 67% with 180-day stratification (Isley, 1944), 20% with 150-day (Keddy and Constable 1986), 50% with 30-day (Keddy and Ellis, 1985), 1-2% with 150-day (Muenscher, 1936), 10% with 270-day (Shiplot and Parent, 1991), and 34% 182-day with bleach scarification (Wagner and Oplinger, 2017). None of these studies compared seed source populations, most having only one or two sources mostly located in the northern-most part of its range (Ontario, Canada, and New York, although Wagner and Oplinger, 2017 were located in Utah). Current land management resources for propagation from seed of *S. pungens* are largely based on studies of closely related species and recommend both scarification and stratification (Stevens et al., 2012). Although chemical stratification and cold stratification have been found to help germination in sedge species, duration and concentrations are highly species dependent, and chemical pre-treatment has been found to adversely affect some species while being vital for others (Rosbakh et al. 2019). Studies done on the *Schoenoplectus* genus found that bleach scarification improved germination in *Schoenoplectus lacustris* (3-4% for 3 days, 80% germination, Clevering 1995) while other studies found that bleach scarification combined with cold stratification improved germination in *Schoenoplectus americanus* (bleach 0.5% for 90 hours, 12 months at 4C, 35% germination) and *Schoenoplectus acutus* (0.05% for 95 hours, 12 months at 4C, 37%, Wagner and Oplinger, 2017). Thullen and Eberts (1995) found two weeks of stratification improved germination of *S. acutus* but that the highest germination rate of 97.5% in those seeds germinated under a temperature regime fluctuating between 10 and 25 C following a 12 week cold stratification, with no difference resulting from population between two seed sources. The relatively low concentration of bleach used may indicate that the seed coat needs minimal scarification and that cold stratification is of more importance for breaking dormancy. Schütz and Rave (1999) found that *Carex* species are most likely spring germinators, and in their study of 32 *Carex* found that in addition to cold-wet stratification, germination was improved by higher temperature and light levels, and temperature fluctuations. These requirements for dormancy break may ensure that germination occurs later in spring when seeds can take advantage of gaps created by late spring disturbances, a strategy that may also influence *S. pungens* coastal populations which inhabit a high energy environment prone to damage from storm surges, erosion, burial, and bank collapse (Albert et al. 2013). Seedlings may have difficulty establishing in or near existing stands due to their inability to compete for light

(Eriksson, 1992) and may contribute to findings of increased germination within the genera *Carex* and *Scirpus* in light gaps (Grime et al., 1981; Schütz and Rave, 1999).

Seeds from both sites located in Oregon (Coos Bay and Necanicum River) and Gray's Harbor, WA had significantly higher estimated mean percent germination in the cold wet stratification treatment compared to warm dry or cold dry stratification treatments. Interestingly, results from these sites suggests that a period of dry chilling is not sufficient, and that wet chilling is needed to improve germination. In other words, periods of cold temperature did not impact dormancy break or change percent germination when seeds were kept dry.

Skagit River, WA and the Californian sites present interesting bookends to the aforementioned sites. Percent germination for seeds from Skagit River, WA showed no difference between the wet or dry cold stratification methods, but both cold stratification methods resulted in higher germination than in warm dry stratification. This suggests that a period of chilling, regardless of moisture, is more important for seeds from Skagit River, WA. Perhaps this is connected to the increased possibility of freezing (resulting in cold and dry conditions) in Skagit River, WA as opposed to the more temperate coastal Gray's Harbor, WA or more southern and coastal Necanicum River, WA and Coos Bay, OR. In contrast to all other sites, seeds from the two Californian sites, Lake Earl and Big Lagoon, had much higher percent germination following warm dry stratification. Big Lagoon had no difference in percent germination between stratification methods. Lake Earl showed a small improvement of germination in cold wet compared to cold dry stratification, but percent germination following both cold stratification treatments did not differ from that following warm dry.

Seeds from the sites located in California performed equally well following warm and dry stratification as cold dry and cold wet, suggesting that seeds coming from warmer and drier sites may not be as dependent on cold and wet stratification for successful germination (Appendix I). A review of shrubs and trees by Tweddle et al. (2003) suggest that species from arid, very cold, and highly seasonal environments produce seed that is unlikely to be desiccation sensitive and studies have shown intraspecies variation that reflects the micro-habitat which they inhabit. Additionally, those species with seed dormancy showed 90% desiccation tolerance compared with 65% of seeds without dormancy. Sensitivity to desiccation is an important factor

in determining long-term storage potential in the laboratory as well as in the seed bank, as many seeds rapidly lose viability upon drying (Tweddle et al. 2003). Results from this germination study confirm this analysis, showing seed tolerance to desiccation in the California populations of *S. pungens* subjected to high variability and potential for dry-down in situ.

2.6 CONCLUSION

This study suggests that success in propagation from seed in greenhouse conditions for use in wetland restoration is entirely possible for *Schoenoplectus pungens*, but depends on the site of origin of seeds and stratification method used. The percent germination ranged from a low of roughly 0% (Skagit River, WA) following warm dry stratification to a high of roughly 76% (Big Lagoon, CA following warm dry stratification and Skagit River, WA following cold wet stratification). The extreme variability of coastal habitats and the highly stochastic nature of dispersal events may have contributed to the evolution of highly specialized dormancy-break mechanisms in *S. pungens*, leading to relatively low germination rates overall at a given time and condition. The results suggest that implementation of wet and cold stratification is the most consistent method to yield higher germination, if site of origin is unknown. The results suggest that there may be a genetically based latitudinal gradient involved in stratification preference or an effect of local adaptation to winter conditions. Seeds taken from more southern areas on the coast with warmer winter temperatures may perform better using warm and dry stratification. Seeds from more northern areas which experience colder winter conditions may perform better in cold wet stratification. Ultimately, higher germination rates could be achieved by tailoring stratification to population preferences. Future studies may improve germination of *S. pungens* by including bleach scarification combined with temperature and light fluctuation. Furthermore, the interannual variability in seed production observed in the field shows that seed cohort dynamics may be influencing low germination in certain sites, with possible variability in dormancy-break requirements within one cohort of produced seeds. Future studies should focus on teasing apart dormancy types and mechanisms in *S. pungens* to improve germination for restoration and to expand our understanding of seed physiology and seed dynamics of wetland species important for creating wildlife and fish habitat.

Results from this study will help with germination under greenhouse conditions but improving success of field sowing will have a substantial impact on restoration potential. High germination from some source populations and the 100% survivorship to reproducing adult stage in fresh and brackish greenhouse conditions (excluding Coos Bay population, Tuttle, forthcoming) imply that failure of field sown seeds is most likely due to conditions found *in situ*, including but not limited to salinity, predation, seed transport, elevation, desiccation, and burial. The current study does not provide convincing evidence for an absence of variability in seed viability between sites and should be investigated in future studies. The local population genetics and reproduction dynamics were outside the scope of this investigation but, observationally, the amount of fertile material in the field was highly variable. Patch size and effective population size may impact germination results and should be investigated further.

**3 - ECOTYPIC VARIATION IN GROWTH AND REPRODUCTIVE
PLASTICITY OF *SCHOENOPLECTUS PUNGENS* (VAHL) PALLA UNDER
VARYING INUNDATION AND SALINITY TREATMENTS**

3.1 INTRODUCTION

Canonical studies in population ecology underscore the importance of genetic diversity and large effective population size on local adaptation and population fitness. Local adaptation has been found to be positively correlated to effective population size suggesting the impact of genetic diversity and gene flow on fitness (Leimu and Fischer, 2008). Extinction due to inbreeding and genetic drift have been found to be linked to smaller effective population size (Newman and Pilson, 2017). Some of these foundational concepts seemingly contradict strategies of monotype-forming clonal wetland species in which vegetative propagation is the main mode of local population growth (Stebbins 1950; Silander 1985). Although many clonal plant species can produce offspring through both clonal propagation (asexual reproduction) and sexual reproduction, in aquatic and emergent angiosperms asexual clonal modes of reproduction are said to predominate over sexual modes (Grace 1993, Sculthorpe). Limited sexual reproduction reduces both recombination and genetic diversity (Barrett, Eckert, & Husband, 1993).

A clonal growth strategy can mean that increasing ramet density may result in the loss of genets through intra-specific competition, therefore expanding populations can show decreasing genetic diversity (Hutchinson 1975, van der Maarel, 2005). Studies have demonstrated that clonal species can form entire geographically isolated patches made up of one genotype (De Greef & Triest, 1999). Genets with many ramets may swamp sexual reproduction when it does eventually occur (van der Maarel, 2005, Shumway and Bertness, 1992) and clumped clonal growth may increase the rate of geitonogamous pollination thereby risking fitness reduction (Charpentier, 2001). These findings suggest that clonal growth would lead to low genotypic diversity, small effective populations, and little to no recombination from sexual reproduction (or at least minimal outcrossing and higher selfing) which in turn could result in lower fitness and lower probability of persistence (Vange, 2002). And yet, clonal growth forms are highly successful judging from their distribution and abundance in highly variable environments (Santamaría, 2002; Roman & Darling, 2007; Sosnová et al., 2010; Sosnová, et al. 2011).

A possible answer lies in the potential of phenotypic plasticity which acts to buffer genotypes in variable environments and reduce selection pressure (Barrett et al., 1993). Prolific clonal propagation forming genetically uniform but phenotypically plastic populations would reduce the risk to the given genotype (Sultan, 1995; van Kleunen & Fischer, 2001). Phenotypic plasticity has been studied extensively in wetland species as a mechanism of distribution by either increasing resource acquisition or acclimatizing to stress in a highly variable environment (Dorken and Barrett 2004). Phenotypic variation of continuous traits in other wetland species has been found to be correlated with environmental factors as well: in response to water level (Lieffer and Shay, 1981; Clevering and Hundscheid, 1998; Seliskar 1990), environmental gradients (Seliskar, 1983, 1985a, 1985b, Wilson 1991, Pai & McCarthy, 2005; Richards et al. 2005;), sedimentation (Seliskar, 1990; Li and Xie 2009), geographic origin (Hansen 2007), and mechanical stress/severing (Poor et al. 2005; Puijalon et al. 2008). Therefore, phenotypic plasticity in clonal wetland plants might be expected to be high and between genotype fitness differences to be low, leading to similar response parameters regardless of source population.

A further possibility is local adaptation or ecotypic variation, with dominant individuals being highly successful in their current environment through selective pressure. This possibility would result in fitness differences between ecotypes under specific conditions. Local adaptation to specific environmental regimes or environments is possible in response to both differences in microhabitat conditions (Galen et al. 1991; Sork et al. 1993) and in response to large environmental differences, eg. saline versus freshwater environments (Thompson et al. 1991). Local adaptation may mean that sexual reproduction may play a highly stochastic but nonetheless important role in both colonization and introduction of new genotypes. Although some species are able to spread clonally over long distances (Fahrig et al. 1994), seeds are considered the primary means of long-distance dispersal and may play an important role in species persistence at a meta-population level (Olivieri et al. 1995; Husband & Barrett 1996). There may be inherent trade-offs between asexual propagation and sexual reproduction in clonal plants that are not apparent in annual plants or non-clonal perennials (Gardner & Mang).

In reality, these two possibilities are not mutually exclusive and both may play a role in population dynamics. Nonetheless, some important implications could result from populations that favor one strategy over another. For example, clonal populations that favor local adaptation

and have high clonal reproduction, with low sexual reproduction of few genets may be more susceptible to sudden and dramatic changes in environmental conditions. With changing climatic conditions that could impact the leading and exposed edges of coastal marshes, it is important to understand the role of morphologic plasticity and reproductive strategy in a plant's resilience to heterogeneous environmental conditions and altered disturbance regimes.

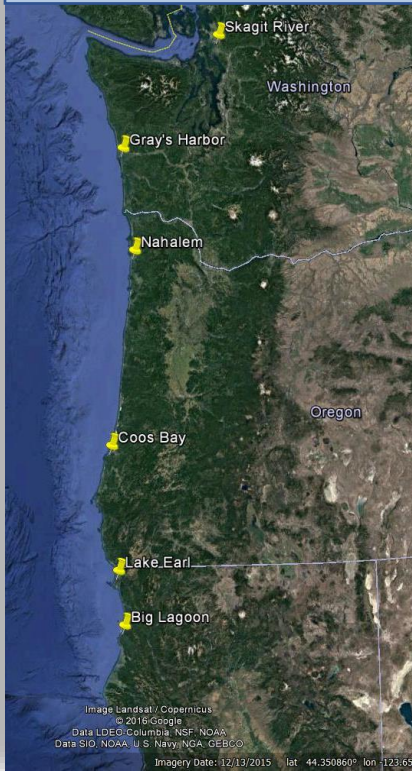
Gradients in salinity and inundation are major factors responsible for zonation of vegetation communities in coastal wetlands, from the Atlantic, Pacific, and Gulf coasts (Howard and Mendelssohn, 1999). Tidal cycles, flooding, and storm surges can all lead to high variability in salinity and inundation in these environments and it is important to understand the vegetative response to these forces. Recent studies have found morphologic variation of *S. pungens* between wetland zones in populations in Tillamook Bay, OR and Cecil Bay, MI (Albert et al., 2013). Parameters assessed and found to show morphological differences included total biomass, below-ground biomass, above-ground biomass, stem density, stem height, stem diameter, a flexibility (Tillamook Bay, OR only), and aerenchyma (Albert et al., 2013). These factors were suggested to increase fitness in high wave energy and depositional environments. These morphological differences show a vegetative response to changing abiotic factors but whether these are due to local adaptation or plasticity would require paired greenhouse or mesocosm experiments to determine.

Previous studies of salt-marsh species have found variation in salinity tolerance between populations (Silander 1985; Allen et al. 1997; Hester et al. 1998; Seliskar & Gallagher 2000), which may indicate the evolution of salt-adapted ecotypes. Many of these plant species that occur in saline habitats have been found to reproduce primarily via rhizomes or other clonal structures (Silander 1985; Mitsch & Gosselink 1993), although they maintain the ability to reproduce sexually (Silander 1985). The relatively low occurrence of sexual reproduction in these species has been attributed to reduced germination (Shumway & Bertness 1992) or high seedling mortality (Abrahamson 1980; Ungar 1991), but may also be due to the difficulty of studying seed dynamics in these habitats. For example, studying seed dynamics of Cyperaceae in wetlands presents many challenges, from tiny seeds, high interannual, intra- and interspecific, and intrapopulation variation in seed production, and difficulty in tracking and identifying seeds and seedlings (see germination chapter). Although poorly understood in wetland plants,

reproductive plasticity would allow shifts in allocation between sexual or asexual propagules under changing salinity conditions (Adam 1990). Analytical and simulation models have been developed to predict the facultative, plastic responses of clonal plants to changing environmental conditions (Loehle 1987; Sakai 1995; Gardner & Mangel 1999). All three models predict that seed production should be favored when the probability of recruitment and establishment is greater than that of asexual ramets, i.e. if the threat of mortality to the ramet is sufficiently high.

The primary questions of interest in this study is whether different populations of *Schoenopletus pungens* show variation in response to salinity and inundation which would in turn be indicative of local ecotypic adaptation. Findings of significant interactions between salinity, inundation, and source population would provide evidence for differential adaptation, with certain populations performing better than others in growth or reproductive traits. Conversely, no between-site differences in response to salinity and inundation may support the interpretation of phenotypically plastic responses. A further question centers on the reproductive response to changes in salinity and inundation. Trade-offs between culm and seed production under varying abiotic conditions could be indicative of changes in allocation between sexual and asexual reproduction. Experimentally, this is done using a reciprocal planting with individuals from the same population, thereby effectively equalizing the environmental variation. The proposed study is an attempt to reconcile seeming contradictions between the dominance of clonal reproduction but the advantage of genetic diversity through sexual reproduction.

Figure 3.1: Map showing study sites of *S. pungens*



3.2 METHODS:

Seeds were collected in late summer of 2015 from 6 different sites: 1) Big Lagoon, CA, 2) Lake Earl, CA, 3) Coos Bay, OR, 4) Necanicum River, OR, 5) Gray's Harbor WA, and 6) Skagit River, WA (Figure 3.1). Detailed methods of stratification and germination can be found in the previous chapter. A common garden experiment was conducted at Oregon State University in Corvallis, Oregon using two concurrent randomized block designs with three factors: salinity (three levels: fresh – 0 ppt, brackish – 15ppt, and high salt - 30ppt), inundation (two levels: tidal and non-tidal), and site of origin (Appendix II for experimental design).

Once in the outdoor mesocosm, seedlings were given an establishment period in freshwater of one month prior to the first addition of salt. All salinity treatments were housed next to each other outdoors in a south-facing lot protected from strong winds between two greenhouses. Shade cloth (50%) was used during midsummer as the Willamette Valley can exceed temperatures and hours of intense sun found at any of the coastal sites of origin. The shade cloth helped control for any sun or temperature stress that could affect the results.

The non-tidal experiment contained 90 total plants in individual 6in (15 cm) diameter pots distributed between 15 clear plastic totes. Each tote was filled to six inches with water from the same tanks used for the tidal treatment. The level of water, which resulted in 5 cm of inundation of the above-ground portion of the plant, was maintained throughout the experiment by addition of water as necessary. Each tote contained 6 pots, one pot from each site of origin. Each tote was randomly assigned a salinity treatment, with 5 totes in each of the three salinity treatments. This resulted in 30 pots per salinity treatment and each site having 15 total replicates, with 5 replicates in each salinity treatment. Inundation was adjusted to be three

inches (7.5 cm) above the pot surface at “high tide” and to be 3cm above the bottom of the pot at “low tide”, for a total daily fluctuation of 24cm. Water was drained and filled once daily, resulting in a “diurnal tidal cycle”. Response parameters during 2017 growing season included number of live culms per pot, maximum culm height, and fertile culms per pot.

3.2.1 Statistical Methods:

Generalized linear mixed models (GLMM) were fit to the data using R package “glmmTMB” (Brooks et al., 2017). Models were chosen based on the *a priori* experimental design and the lowest AIC. Model diagnostics, including simulated residuals, dispersion tests, and tests for zero inflation, were done using R package “DHARMa” (Hartig, 2021). Marginal means and multiple pairwise comparisons were estimated using the “emmeans” package in R (Lenth, 2021). Plots were constructed using R package “ggplot2” (Wickham, 2016).

3.3 RESULTS:

Unexpectedly, the first year of growth produced no fertile inflorescences across the entire experiment. Inflorescence scars were visible on some culms but no inflorescence was produced on any culms.

3.3.1 Proportion of fertile culms:

The GLMM fitted to the proportion of fertile culms included pot ID as a random effect and salinity, inundation, site, and month as fixed effects. Two-way interactions between salinity x inundation, salinity x month, inundation x site, and inundation x month were included and resulted in the lowest AIC. The model used a beta distribution and a zero-inflation correction for salinity. Model diagnostics showed no overdispersion or zero-inflation for the final model.

The significant differences between mean proportion of fertile culms by month ranged between 0.06 and 0.12, by inundation conditions ranged between 0.13 and 0.28, by site ranged between 0.10 and 0.50, and by salinity ranged between 0.07 and 0.54 (Appendix III: Table III.4).

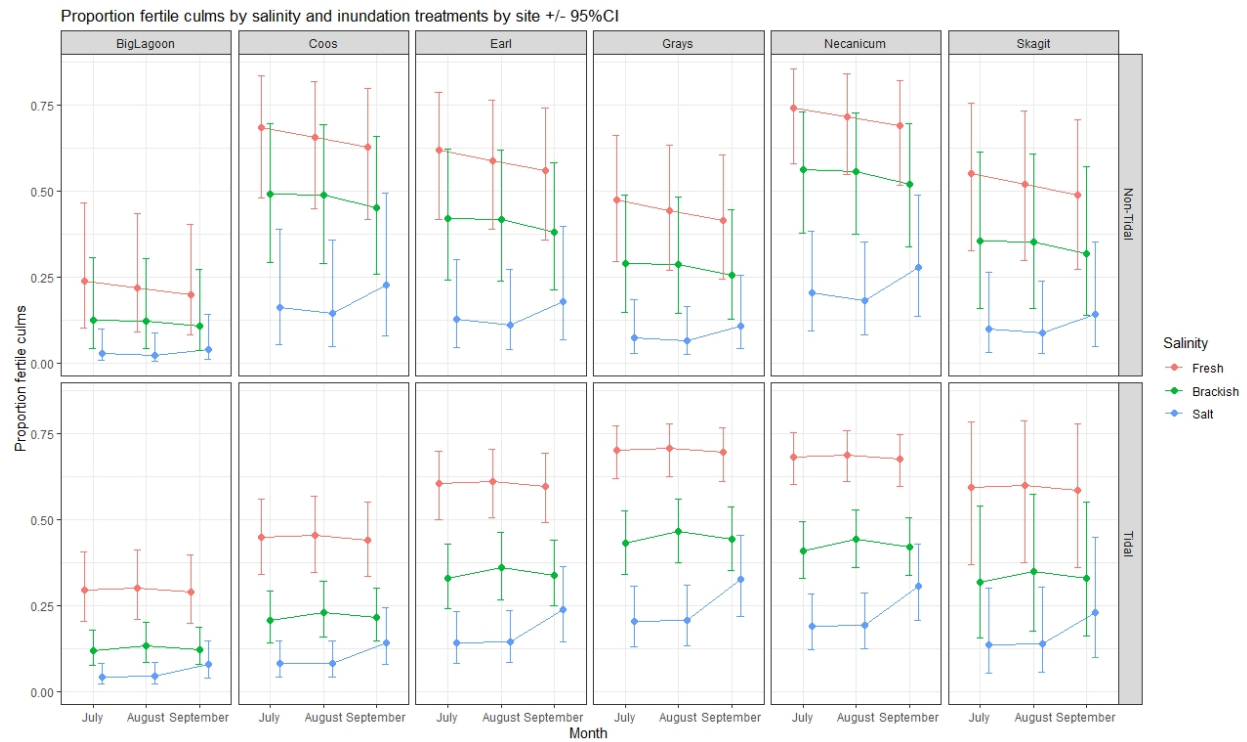


Figure 3.2: Graphs of the estimated marginal means ($\pm$ 95% CI) of the proportion of fertile culms per pot by *Schoenoplectus pungens* individuals from six geographically distinct source populations under varying salinity treatments and inundation conditions.

Across all sites, months, and in both inundation conditions, a significantly higher proportion of culms produced fertile material in the fresh-water treatment compared with salt (Figure 3.2, Table 3.1, Appendix III: Table III.4, $p < 0.05$ for all within site comparisons with Bonferroni adjustment, except Big Lagoon, CA in September non-tidal). Differences between fresh and brackish treatment varied by inundation conditions, with no difference in the proportion of fertile culms between the fresh and brackish treatment in the non-tidal condition, regardless of month or site (Figure 3.2, Table 3.1, Appendix III: Table III.4, $p > 0.05$ for all within site comparisons with Bonferroni adjustment). In contrast, in the tidal condition the proportion of fertile culms was higher in the fresh treatment than the brackish for all sites and all months (Figure 3.2, Table 3.1, Appendix III: Table III.4, $p < 0.05$ for all comparisons with Bonferroni adjustment).

Table 3.1: Summary comparisons in estimated marginal means of the proportion of fertile culms per pot by *Schoenoplectus pungens* individuals from six geographically distinct source populations under varying salinity treatments and inundation conditions. All comparisons were conducted using all grouping variables. If a grouping variable was not found to differ in estimate, it was collapsed for this table. See Appendix 1 for numerical values of the comparison results.

A. Between Sites:							
Contrast	Inundation	Fresh	Brackish	Salt			
BigLagoon < Coos	Non-Tidal	*	*				
BigLagoon < Necanicum	Non-Tidal	*	*				
BigLagoon < Earl	Tidal	*	*	*			
BigLagoon < Grays	Tidal	*	*	*			
BigLagoon < Necanicum	Tidal	*	*	*			
Coos < Grays	Tidal	*	*	*			
Coos < Necanicum	Tidal	*	*	*			
B. Between Months:							
Contrast	Inundation	Fresh	Brackish	Salt			
August < September	Non-Tidal			* (Only Necanicum)			
July > September		* (Except Big Lagoon)					
August < September	Tidal			*			
July < September				*			
C. Between Inundation:							
Contrast	Site	Fresh	Brackish	Salt			
(Non-Tidal) > Tidal	Coos	*(July)	*				
(Non-Tidal) < Tidal	Grays	*	* (Sept.)	*			
D. Between Salinity:							
Contrast	Inundation	Site:					
		Big Lagoon	Earl	Coos	Necanicum	Gray's	Skagit
Brackish > Salt	Non-Tidal		*	*	*	*	*
Fresh > Salt		*	***	***	***	***	***
Brackish > Salt	Tidal	*	*	*	*	*	*
Fresh > Brackish		***	***	***	***	***	***
Fresh > Salt		***	***	***	***	***	***

* = July and August

*** = All months

Differences between salt and brackish varied by month, with a significantly higher proportion of fertile culms produced in brackish than salt in both July and August and in both non-tidal and tidal conditions at all sites except Big Lagoon, CA in non-tidal conditions ($p < 0.05$ for all significant comparisons with Bonferroni adjustment). No significant difference in proportion of fertile culms in the month of September was found ($p < 0.05$ for all within site x salinity comparisons in July and August, $p > 0.05$ for all within site x salinity comparisons in September with Bonferroni adjustment).

Differences in the proportion of fertile culms between inundation conditions compared within site, month, and salinity, were only apparent in certain months x salinity of Coos Bay, OR and Gray's Harbor, WA (Appendix III: Table III.4, $p < 0.05$ for significant comparisons with Bonferroni adjustment). Significant comparisons showed that Gray's Harbor, WA had a higher proportion of fertile culms in tidal conditions than non-tidal conditions whereas Coos Bay, OR had the opposite trend.

Between site differences were limited to Big Lagoon, CA in both tidal and non-tidal conditions and Coos Bay, OR in tidal conditions. In non-tidal conditions, Big Lagoon, CA produced a smaller proportion of fertile culms than Coos Bay, OR and Necanicum River, OR in fresh and brackish treatments (Table 3.1, Appendix III: Table III.4, $p < 0.05$ for significant comparisons with Bonferroni adjustment). In tidal conditions across all salinity treatments, Coos Bay, OR produced a lower proportion of fertile culms than both Gray's Harbor, WA and Necanicum River, WA; as did Big Lagoon, CA with the addition of Lake Earl, CA (Appendix III: Table III.4, $p < 0.05$ for significant comparisons with Bonferroni adjustment).

3.3.2 Live culms per pot:

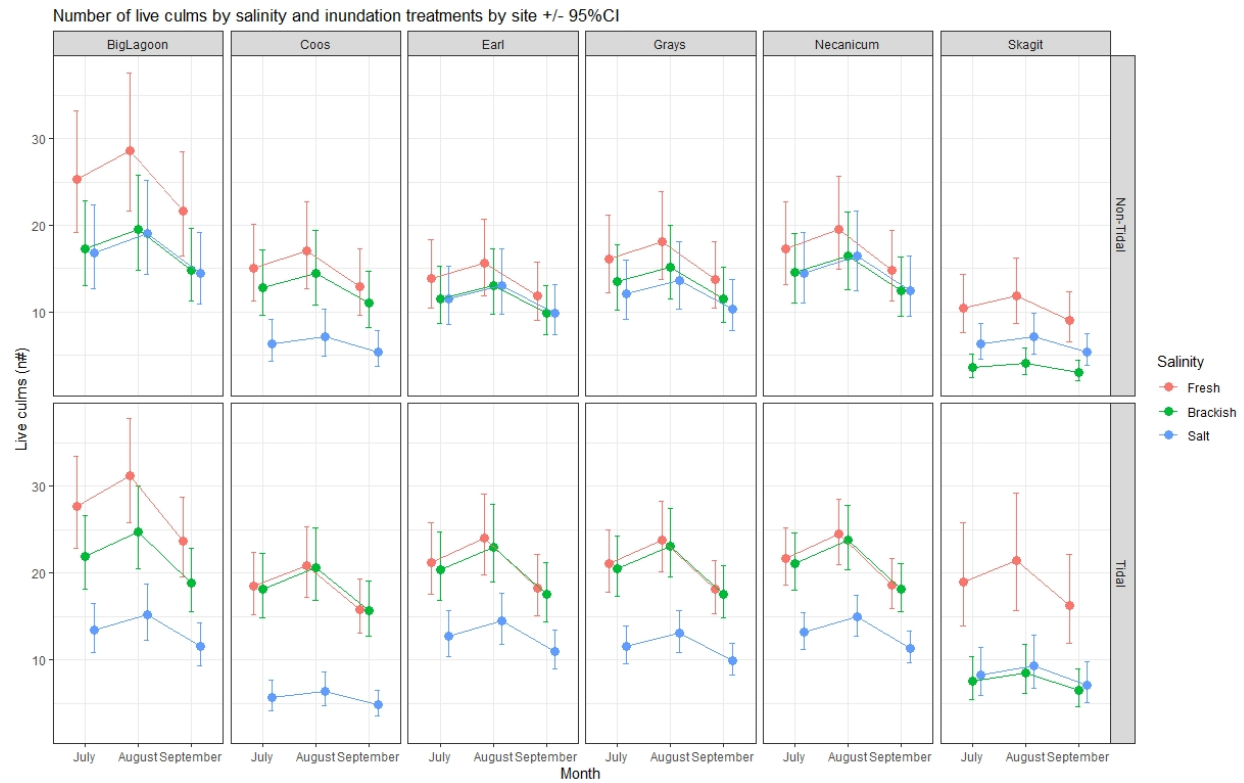


Figure 3.3: The estimated marginal means ($\pm$ 95% CI) of live culms produced per pot by *Schoenoplectus pungens* individuals from six geographically distinct source populations under varying salinity treatments and inundation conditions.

A. Between Sites:				
Contrast	Inundation	Fresh	Brackish	Salt
BigLagoon > Earl	Non-Tidal	*		
Coos < BigLagoon		*		*
Coos < Earl				*
Coos < Grays				*
Coos < Necanicum				*
Skagit < BigLagoon		*	*	*
Skagit < Coos			*	
Skagit < Earl			*	*
Skagit < Grays			*	*
Skagit < Necanicum			*	*
Coos < BigLagoon	Tidal	*		*
Coos < Earl				*
Coos < Grays				*
Coos < Necanicum				*
Skagit < BigLagoon			*	*
Skagit < Coos			*	
Skagit < Earl			*	*
Skagit < Grays			*	
Skagit < Necanicum			*	*

B. Between Months:			
Contrast	Fresh	Brackish	Salt
August > September	*	*	*
July < August	*	*	*
July > September	*	*	*

C. Between inundation:				
Contrast	Site	Fresh	Brackish	Salt
Non-Tidal < Tidal	Earl	*	*	
Non-Tidal < Tidal	Grays		*	
Non-Tidal < Tidal	Necanicum		*	
Non-Tidal < Tidal	Skagit	*	*	

D. Between salinity:							
Contrast	Inundation	Site:					
		Big Lagoon	Earl	Coos	Necanicum	Gray's	Skagit
Brackish > Salt	Non-Tidal			*			
Fresh > Brackish							*
Fresh > Salt				*			
Brackish > Salt	Tidal	*	*	*	*	*	
Fresh > Brackish							*

Table 3.2: Simplified comparisons in estimated marginal means of live culms produced per pot by *Schoenoplectus pungens* individuals from six geographically distinct source populations under varying salinity treatments and inundation conditions. All comparisons were conducted using all grouping variables. If a grouping variable was not found to influence the comparison, it was collapsed for this table. See Appendix 1 for numerical values of the comparison results

Fresh > Salt		*	*	*	*	*	*
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The GLMM fitted to the mean live culms per pot included pot ID as a random effect and salinity, inundation, site, and month as fixed effects. Two-way interactions between salinity x inundation and salinity x site were included and resulted in the lowest AIC. The model used a poisson distribution and a zero-inflation correction for salinity and site. Model diagnostics showed no overdispersion or zero-inflation for the final model. The significant differences between mean live culms by month ranged between 3.60 and 7.53, by inundation conditions ranged between 3.31 and 9.87, by salinity ranged between 5.89 and 16.23, and by site ranged between 9.59 and 16.35, (Appendix III: Table III.2).

Live culms per pot differed significantly between fresh and salt treatments for inundation conditions and site. In the tidal condition, the number of live culms was higher in the fresh treatment than in the salt treatment for all sites and all months ($p < 0.05$ for all comparisons with Bonferroni adjustment). Coos Bay, OR was the only site that had a significant difference in mean live culms between fresh and salt treatments in the non-tidal conditions, with more culms in fresh (Figure 3.3, Table 3.2, and Appendix III: Table III.2, $p > 0.05$ for all within-site comparisons with Bonferroni adjustment). Skagit River, WA was the only site to have a significant difference in live culms between fresh and brackish treatments, in both tidal and non-tidal conditions ($p < 0.05$ for all Skagit River, WA comparisons with Bonferroni adjustment).

Differences in live culms per pot between brackish and salt treatments differed by inundation and site, in the case of Skagit River, WA and Coos Bay, OR. All sites except Skagit River, WA had significantly more live culms in the brackish treatment than salt in the tidal condition in all months, but no difference was found in the non-tidal condition (except Coos Bay, OR) (Appendix III: Table III.2). Coos Bay, OR had fewer live culms in brackish than salt in the nontidal treatment as well.

3.3.3 Maximum height

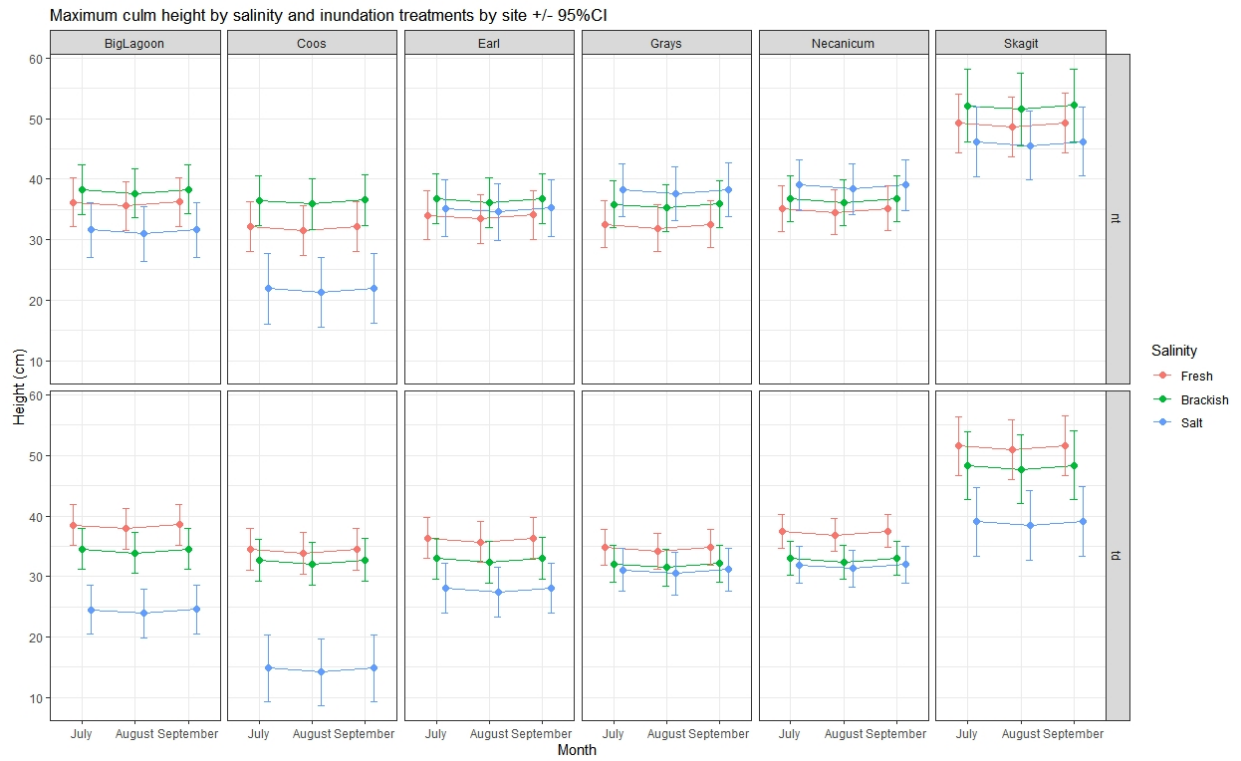


Figure 3.4: The estimated marginal means ($\pm$ 95% CI) of the maximum culm height per pot by *Schoenoplectus pungens* individuals from six geographically distinct source populations under varying salinity treatments and inundation conditions.

Table 3.3: Simplified statistical comparisons of estimated marginal means of live culms produced per pot by *Schoenoplectus pungens* individuals from six geographically distinct source populations under varying salinity treatments and inundation conditions. All comparisons were conducted using all grouping variables. If a grouping variable was not found to influence the comparison, it was collapsed for this table. See Appendix 1 for numerical values of the comparison results.

Comparison Results:

Contrast	Fresh	Brackish	Salt				
A. Between Sites:							
BigLagoon < Necanicum			*				
BigLagoon < Skagit	*	*	*				
Coos < Earl			*				
Coos < Grays			*				
Coos < Necanicum			*				
Coos < Skagit	*	*	*				
Earl < Skagit	*	*	*				
Grays < Skagit	*	*					
Necanicum < Skagit	*	*					
B. Between Months:							
August < September	*	*	*				
July > August	*	*	*				
C. Between inundation:							
Non-tidal > Tidal		*	*				
D. Between salinity:							
Contrast	Inundation	Site: Big Lagoon	Earl	Coos	Necanicum	Gray's	Ska
Brackish > Salt	Non-tidal			*			
Fresh > Salt				*			
Brackish > Salt	Tidal	*		*			
Fresh > Salt		*	*	*	*		*

The GLMM fitted to the mean maximum culm height included pot ID as a random effect and salinity, inundation, site, and month as fixed effects. Two-way interactions between salinity x inundation, salinity x site, and inundation x salinity were included and resulted in the lowest AIC. The model used a gaussian distribution and a zero-inflation correction for site and a dispersion correction for site x salinity. Model diagnostics showed no overdispersion or zero-inflation for the final model. Based on the model, month had no interaction with any other factors and therefore the following comparisons did not vary between months.

The significant differences between mean live culms by month ranged between 0.63cm and 0.65cm, by inundation conditions ranged between 3.77cm and 7.06cm, by salinity ranged

between 5.54cm and 19.63cm, and by site ranged between 7.39cm and 24.24cm (Appendix III: Table III.1). The only effects of month were a slight dip in the mean max culm height in August, July was $0.63\text{cm} \pm 0.4$ 95%CI higher than August, and September was $0.65\text{cm} \pm 0.4$ 95%CI higher than August (Appendix III: Table III.3, $p < 0.05$ for all comparisons).

Site comparisons varied by salinity. Skagit River, WA had higher mean maximum culm height in all salinity and inundation combinations than Big Lagoon, CA, Coos Bay, OR, and Lake Earl, CA (Figure 3.4, Table 3.3, Appendix III: Table III.3, $p < 0.05$ for all comparisons). Skagit River, WA had higher mean maximum culm height than the remaining sites, Gray's Harbor, WA and Necanicum River, OR, in both inundation conditions but only in brackish and fresh treatments (Figure 3.4, Table 3.3, Appendix III: Table III.3, $p < 0.05$ for brackish and fresh comparisons). Both Coos Bay, OR and Big Lagoon, CA had lower mean max culm heights than Necanicum River, OR in both inundation conditions in the salt treatment (Figure 3.4, Table 3.3, Appendix III: Table III.3, $p < 0.05$ for significant comparisons).

Mean maximum culm height was higher in non-tidal conditions than in tidal conditions in brackish (Figure 3.4, Table 3.3, Appendix III: Table III.3, $3.77\text{cm} \pm 3.23$ 95% CI higher) and salt treatments ($7.06\text{cm} \pm 3.60$ 95% CI higher) for all sites and months ($p < 0.05$ for all pairwise comparisons with Bonferroni adjustment). There was no significant difference in mean maximum culm height between tidal and non-tidal conditions in the fresh treatment (Figure 3.4, Table 3.3, Appendix III: Table III.3, $p > 0.05$ for all).

Differences between mean maximum culm height between salinity treatments varied by site and inundation conditions. Under tidal conditions, all sites except Gray's Harbor, WA produced shorter maximum culm height in the salt treatment compared to those in the fresh treatment ($p < 0.05$ for pairwise comparisons with Bonferroni adjustment, $p > 0.05$ for Gray's Harbor, WA pairwise comparisons). The mean maximum culm height in the salt treatment in the tidal conditions were also shorter than the brackish treatment in those plants from Big Lagoons, CA ($9.99\text{cm} \pm 6.38$ 95% CI shorter, $p = 0.0005$) and Coos Bay, OR ($17.87\text{cm} \pm 9.88$ 95% CI shorter, $p < 0.001$). In the non-tidal condition, only Coos Bay, OR showed significant difference between maximum culm height between salinity treatments. Mean maximum culm height was

shorter in the salt treatment than both brackish ($14.58\text{cm} \pm 8.7$ 95% CI shorter, $p = 0.0001$) and fresh treatment ($10.24\text{cm} \pm 8.65$ 95% CI shorter, $p = 0.014$) (Appendix III: Table III.3).

3.4 DISCUSSION:

The results show that salinity, inundation, and source population all impact the proportion of fertile buds, maximum height, and the number of culms produced, although to varying extent. Month did account for significant statistical variation in all models, but from an ecological perspective the differences between months were small. This may suggest that dehiscence and senescence may occur later in the season, and that initial culm growth happens rapidly following emergence in spring. In the tidal-salt treatment, the inverse monthly trends of the number of culms and the proportion of fertile culms suggests that culms bearing inflorescences remained living longer than those that were not fertile.

From this study, it is clear that abiotic factors, including site of origin, are influencing seed production in *S. pungens*. Our results indicate trade-offs between sexual and asexual reproduction with life-stage, in this case first and second year of growth, although further study is required to investigate the nature of these trade-offs. In a study of *Scirpus mariqueter* it was found that asexual reproduction was favored in the early life stages and sexual reproduction was predominant in mature stands (Sun et al., 2013). This may be supported by the lack of fertile material produced during the first year of the current study and the production of fertile material only in the second year.

There are limited data regarding the plastic reproductive strategies of clonal plants exposed to salinity stress. Numerous clonal reproduction models predict that sexual reproduction should be favored when the quality of the local environment decreases or when the threat of mortality to the parent plant (ramet or genet) increases. Many studies have found an inverse relationship between asexual reproduction and sexual reproduction in clonal plants, with increasing levels of stressors being accompanied by a decrease in asexual reproduction in favor of sexual reproduction. In a study of *Scirpus mariqueter* sexual reproduction increased with declining asexual reproduction along an elevational gradient (Sun et al., 2013). In contrast, the clonal perennials *Sporobolus virginicus* and *Hordeum jubatum* tend to decrease sexual reproduction in higher salinity sites (Blits & Gallagher 1991; Wang & Redmann 1996). The decrease of the proportion of fertile culms of *Schoenoplectus pungens* in high salinity suggests that different strategies may emerge when environments become extremely unfavorable. Results

of a previous study looking at high marsh perennial turf species by Shumway and Bertness (1992) showed that seedling recruitment was negatively impacted by high soil salinity. Their conclusion was that the population dynamics of those species in higher soil salinity environments are dominated by clonal growth (Shumway and Bertness, 1992). If *Schoenoplectus pungens* seedlings have similar response to salinity, this may explain the reduction in sexual reproduction with increased salinity rather than conforming to model of increasing sexual reproduction with declining environmental quality. The results of the current study showed that high salinity combined with tidal conditions not only reduces the overall number of culms, but also reduced the proportion of culms producing inflorescences and the total number of fertile buds.

Lieffers and Shay (1981) found that inundation depth of *Scirpus maritimus* var. *paludosus* causes a shift from clonal growth dominance to seed production. At low inundation depth, plants showed a dominance of clonal growth, producing a higher number of shoots, tillers, and more below ground biomass, which was paired with low seed production. With increased water depth, a shift to a seed-producing strategy was seen, with reduced tillers and biomass, but taller shoots and greater seed production. Dry and highly saline treatments resulted in short, mostly non-flowering stems. Shallow water and reduced salinity resulted in stems that were larger and more numerous. Higher inundation and low salinity resulted in the tallest stems and maximum inflorescence but lower stem number (Lieffers and Shay 1982). The current study showed that although fewer culms were produced in the salt treatment, they tended to grow to a similar maximum height as those in fresh and brackish treatments. This suggests that rather than having a stunting effect on culm height, salt stress had a greater impact on the number of culms produced. This may be related to the detrimental effects that a wetland plant would suffer from being shorter than the water depth, thereby limiting oxygen and access to light.

The number of live culms per plant was surprisingly similar for all salinity treatments under non-tidal conditions, in contrast to the distinct negative effect of high salinity under tidal conditions. Although the number of culms in the tidal treatment had no difference between the fresh and brackish treatment (except Skagit River, WA), the proportion of fertile culms was significantly different, with many more fertile stems in the treatment.

The current study showed some evidence of local adaptation as evidenced by between site differences in response to salinity and inundation. Between site differences in response to salinity and inundation were most marked for Coos Bay, OR, Big Lagoon, CA, and Skagit River, CA. Coos Bay, OR seemed to have a lower tolerance to the high salt treatment as it generally had the lowest maximum height and live culm number. Skagit River, WA had taller culms but generally fewer of them, especially in brackish and salt treatments. Lake Earl, CA, Necanicum River, WA, and Gray's Harbor, WA showed virtually no differences between response variables throughout, suggesting that perhaps these sites produced more phenotypically plastic individuals. Interestingly, individuals from certain source populations produced less fertile material than others: Big Lagoon, Coos Bay, and Skagit River all produced fewer fertile spikelets than Lake Earl, CA, Gray's Harbor, WA and Necanicum River, OR in the fresh water treatment in both tidal and non-tidal, and brackish in tidal conditions.

3.5 CONCLUSION:

This study shows that both local adaption and phenotypic plasticity may play a role in vegetative response to salinity and inundation, and that the degree of local adaptation may differ between sites. Further investigation of sites with high ecotypic variation is warranted to understand the possible response of these populations to rapid environmental change. Further, the clear differences in reproductive allocation with increasing environmental stress is cause for continued study. Changing salinity and inundation regimes have been shown to impact biomass, morphology, and phenology of many coastal wetland plants but little research has been done regarding reproduction dynamics, in part due to the assumed low importance of sexual reproduction in many clonal species and the difficulty of *in situ* studies. Understanding reproduction dynamics, even in species with perceived low sexual reproduction, is important from a conservation perspective. While this may be the case the majority of the time, occasional survival of seedlings could be an important source of both colonization of new stands and introduction of genetic variability in existing stands.

**4 - EFFECTS OF SALINITY AND INUNDATION ON BIOMASS PRODUCTION
AND ALLOCATION IN AN OBLIGATE WETLAND MONOCOT,
SCHOENOPLECTUS PUNGENS (VAHL) PALLA**

4.1 INTRODUCTION

Vegetation in coastal wetland environments provides important ecosystem functions including shoreline stabilization, wave attenuation, storm surge protection, sediment accretion, carbon sequestration, and water quality improvement (Howard et al. 2017, Kennish, 2002; Nahlik and Fennessy, 2016; Shepard et al. 2011; Taylor, 2012; Turner, 2004; Zedler, 2001). Many of these functions are related directly or indirectly to primary production. Studies have shown primary production values in wetlands to be among the highest globally (Montague and Wiegert, 1990; Odum and McIvor, 1990) and support important habitat for ecologically, culturally, and economically important species, including salmonids, shellfish, birds, and marine mammals (Emmet et al., 2000). Primary production also influences the resilience, formation, and survival of coastal marshes by contributing to the dynamic maintenance of marsh surface elevation (Alber et al. 2008; Callaway et al. 1997; Kirwan and Guntterspergen, 2012; Kirwan et al. 2016). As a result, effective management of coastal ecosystem requires a better understanding of the factors that influence primary production.

Two important factors that influence primary production in coastal ecosystems are salinity and inundation. Salinity and inundation regimes of coastal environments are predicted to change due to various factors including sea level rise, climate change, elevation shifts, and changing land use patterns (Alber et al., 2008; Barlow and Reichard, 2010; Church et al 2013; IPCC 2014; Morris et al., 2002; Swanson et al. 2014; Titus, 1988; Terray et al. 2012, Telwala et al., 2013). These shifts are particularly important as gradients in salinity and inundation are major factors responsible for zonation of vegetation communities in coastal wetlands (Chapman, 1941; Engels et al., 2011; Ewing, 1986; Howard and Mendelssohn, 1999) as well as seedling germination and establishment (Engels et al. 2011). Increasing salinity may result from sea level rise leading to saltwater intrusion as well as disruption of freshwater inflows due to drought and anthropogenic withdrawals (Barlow and Reichard, 2011; Swanson et al. 2014). Changes in wet-dry cycles resulting from changes in climatic processes may lead to salt accumulation and the “salinization” of wetlands (Brock and Neilson, 2009). Further, increased incidence of drought may lead to hypersalinization and acute marsh dieback, limiting marsh resilience in the face of sea level rise (Hughes et al. 2012). The ability of wetlands to shift spatially in response to climate change is constrained by both anthropogenic and geomorphic factors and is predicted to

lead to an overall shift to more saline wetlands (Parker et al. 2011, Williams et al. 1999; Visser et al. 2013), ultimately resulting in changes in species and community structure and distribution (Janousek and Mayo, 2013; Warren and Niering, 1993; Visser et al. 2002).

Salinity and inundation impact plant physiological processes, and at a broad scale, impact marsh primary productivity. A review by Barrett-Lennard (2003) found that the interaction between inundation and salinity generally leads to adverse effects on plant growth and survival greater than the sum of the impacts taken singly. Increasing saline inundation duration, frequency, and depth may result in decreased plant productivity, decreased organic and sediment accumulation, and ultimately widespread plant death resulting in erosion and loss of coastal marshes (Nyman et al. 2006; Visser and Peterson, 2015). Decreased primary productivity and changes in biomass allocation interrupt the feedback mechanisms that allow for sediment and organic matter entrapment and retention above-ground and rhizome and root zone expansion below-ground and above the marsh surface needed to maintain plant species at elevations that allow persistence (Morris et al., 2002; Nyman et al., 2006). Understanding shifts in biomass production and allocation under varying inundation and salinity conditions will be crucial to allow forecasting of changes in annual net primary production (ANPP), carbon sequestration (Nahlik and Fennessy, 2016), distribution, and ultimately persistence of coastal vegetated marshes (Dugger, 2016; Shepard et al., 2011; Stagg et al. 2017; Thorne et al., 2018).

Salinity is an important stressor dictating plant function, plant physiology, and community resilience in estuarine environments and presents significant challenges for plant growth (Aslam et al., 2011; Buffington et al., 2020; Howard and Medelsohn, 1999; Munns and Tester, 2008; Pennings et al. 2005; Poljakoff-Mayber, 1988). The challenges posed by increasing salt concentrations in the environment and, by extension in plant tissues, is two-fold: osmotic stress and direct toxicity (Munns and Tester, 2008). Numerous researchers have investigated halophytes and their response to salinity including studies on the ecology of halophytes (Reimold, and Queen, 1974), comparative physiology (Munns, 2002), mechanisms of salinity tolerance (Munns and Tester, 2008), ecophysiology (Rozema et al. 1985), response of the *Chenopodiaceae* (Grigor and Toma, 2007), and a general review (Aslam et al. 2011). Halophytes, defined as tolerant of $>200\text{mM}$, or roughly one third the salinity of seawater, account for roughly 1% of the world's flora; with only 0.25 % of flowering plant species known

to be able to complete their life cycle in saline conditions (for reference, seawater has a salinity of about 3.5%, 35 g/L, or 599 mM) (Flowers & Colmer, 2008; Flowers et al., 2010). The relatively small number of halophytic plants paired with their disproportionate importance in coastal ecosystems, underlines the importance of understanding the dynamics and range of tolerance of this vital group of plants in the face of potentially changing salinity and inundation regimes.

Prolonged periods of flooding often go hand in hand with increased salinity and are an important abiotic factor in determining distribution and zonation of vegetation in coastal wetlands (Barrett-Lennard, 2003; Colmer and Voesenek, 2009; Flowers and Colmer, 2008). Flooding causes soil hypoxia and anaerobic conditions by filling soil pore spaces, impacting plant function as well as reducing shoot photosynthesis in submerged tissue (Flowers and Colmer, 2008). Soil hypoxia can result in decreased oxygen concentrations in plant tissue which can lead to reduction of metabolic processes and long-term morphologic changes (Geigeneberger 2003; Batool et al. 2013). Structural adaptation in vascular plants that act to counter anoxia include the development of pore space in cortical tissues, which allows oxygen to diffuse from the aerial parts of the plant to the roots to supply root respiratory demands (Flowers and Colmer, 2008).

Although past studies have sought to investigate the interaction between salinity and inundation, few have included the possibility of ecotypic variation in response to these factors. Due to legitimate logistical limitations, most plant ecology studies are conducted at fine geographic scales, at one site or with material from a single population (Pennings et al. 2003). These data are then generalized to broad geographic, species or population scale. However, broad geographic scale encompasses vast variability in abiotic factors and in clonal species or in populations with low gene flow, generalization may overlook important interspecific variation and ecotypic variation (Pennings et al. 2003). One obstacle to constructing such generalities is that the nature of interactions among species may change with abiotic conditions (Dunson and Travis 1991). West coast estuaries are geomorphically varied and range from deep fjords to small, shallow lagoons with associated variations in hydrology and salinity regimes (Emmet et al., 2000). These differences may translate to ecotypic variation in plant species that occur across its range, similar to what has been observed on the Atlantic coast where southern

populations showed differing salinity tolerances to northern populations of the same species (Pennings et al., 2003).

The current study seeks to investigate the impacts of salinity and inundation on biomass production and allocation of a cosmopolitan wetland monocot, *Schoenoplectus pungens*, with seeds taken from six Pacific Coast populations ranging from northern California through Oregon and Washington. Questions that will be investigated in this study include: 1) Does biomass production and biomass allocation (RSR) in *S. pungens* differ depending on salinity, inundation regime, and site of origin? 2) Does the effect of salinity on biomass differ with inundation? 3) Does the relationship between biomass, salinity, and inundation differ with site of origin?

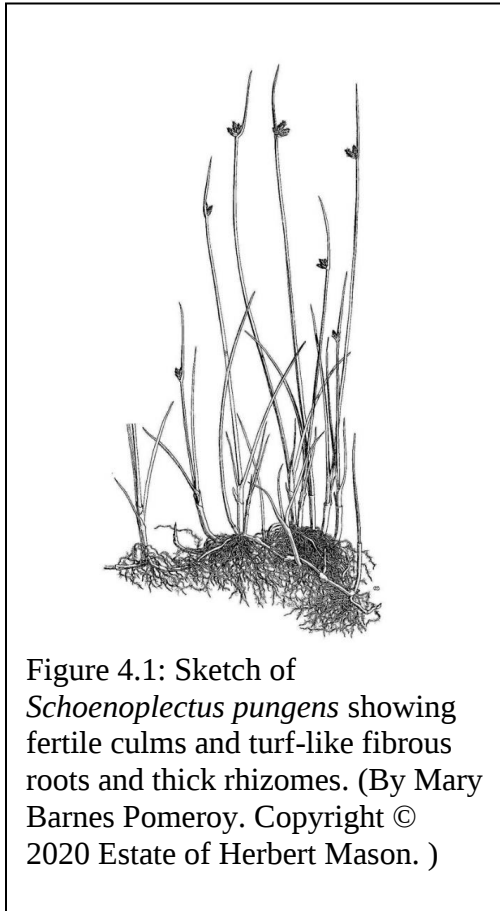
S. pungens was chosen as a model organism for studying biomass dynamics of salt-tolerant clonal monocots as the clonal growth form is prevalent in coastal and estuarine systems, often being a foundational functional group. The study builds upon and expands on previous work focusing on wave attenuation (Yoon et al. 2011) and sedimentation and erosion control by *S. pungens* (Lemein dissertation). *Schoenoplectus pungens*, also known as common threesquare, sweetgrass, bulrush beach grass, sweet grass, American three-square, common threesquare, bulrush, three-cornered grass, and tule, is an important species that provides habitat for a variety of wildlife, as well as culturally irreplaceable ethnobotanical uses in portions of its range, specifically by the Quinalt, Chehalis, and Skokomish tribes among others (Ryan, 2000). *S. pungens* is a rhizomatous perennial wetland obligate monocot which is widely distributed over a large latitudinal gradient throughout North America and provides important ecosystem services including wave attenuation and shoreline stabilization (Albert et al., 2013).

Total biomass production and biomass allocation patterns impact both ecosystem functions and physiological processes and have not been studied for *S. pungens*. The primary structures of *S. pungens* consist of the above-ground culm, leaves, and inflorescence, and below-ground rhizomes and roots (Figure 1). Above-ground biomass of *S. pungens* has been shown to effectively attenuate wave action when on the leading edge of shorelines and marshes (Yoon 2011). Generally, the below-ground portion of the plant has been described in the literature as comprised of a thick matrix of rhizomes and fibrous roots, similar to a turf, which aides in erosion control and shoreline stabilization along open coast, as well as stream channels and

stream banks (Ikegami et al., 2008, Albert et al., 2013). However, below-ground biomass has been observed to vary widely in ratios of lateral versus vertical rhizomes, internode lengths in rhizomes, proliferation of fibrous roots, aerenchyma formation, color (indicating redox and mineral use), etc. (Albert et al., 2013; Ikegami et al. 2008). It is unknown what the driving factors of these differences in morphology are, but this study hopes to begin to understand broad trends in biomass production and allocation in response to inundation and salinity.

Information regarding salinity and inundation tolerance of *S. pungens* is based on its distribution and location within wetland elevational gradients but has not been explicitly investigated in lab or greenhouse settings. *S. pungens* is assumed to be highly tolerant of inundation and has regularly been found growing at depths of 120 cm, but also grows on coastal dunes where the water table is more than 1m below the soil surface, and the plant is also tolerant of fluctuating water levels during the growing season (Albert, personal communications and Great Lakes Restoration Initiative field notes from 2011-2020). The salinity tolerance of *S. pungens* has only been studied in the field, linking species presence and distribution to pore water and water column salinity and has been assumed to be highly tolerant of high salinity, nearing full seawater. This assumption is most likely due to *S. pungens* inhabiting coastal areas that receive full seawater inundation at high tide, leading to the assumption that pore water salinity must be that of seawater. Observations following two years of surveys along the Pacific Coast led to the hypothesis that *S. pungens* may be less tolerant to high salinity than previously assumed and underlines the importance of the direct study of biomass response to salinity in light of the possibility of shifting salinity regimes in coastal systems.

S. pungens has a complex taxonomic history, previously known as *Scirpus americanus* Persoon, misapplied as *Schoenoplectus americanus* (Persoon) Volkart ex Schinz & R. Keller, and synonymous with *Schoenoplectus olneyi* (A. Gray) (Smith, 1995). Three varieties, var. *pungens*, var. *longispicatus*, and var. *badius*, have been proposed but are not recognized as subspecies, although the North American Pacific Coast *S. pungens* seems to constitute a distinct morphological group (Smith, 1995) and ongoing genetic studies may result in new nomenclature (Sheils et al. 2014). The varying morphology observed and possibility of a Pacific Coast variety, led to the question of the possibility of variation in plant response among populations to environmental factors resulting in the inclusion of six Pacific Coast populations.



Presented here are the results of biomass harvest of *S. pungens* following two growing seasons. Methods will describe the sampling done in the field at the time of seed collection, followed by detailed description of the construction of the mesocosms used in the common garden experiment, and lastly the statistical methods. The results include the following response variables: above-, below-, and total biomass as well as a calculated root-to-shoot ratio. Differences in these response variables between source populations will indicate ecotypic differences between populations. Interactions between salinity treatments and inundation condition will show whether daily drying effects salinity tolerance of *S. pungens*.

4.2 METHODS

4.2.1 Field Sampling:

Current occurrence and distribution of *S. pungens* in coastal and estuarine wetlands along the Pacific Coast of California, Oregon, and Washington was surveyed between June and September of 2014 and 2015. The occurrence of *S. pungens* in tidally influenced habitats was of interest. Historical herbarium samples, correspondence with current land managers, and aerial photographs were used as starting points to determine areas most likely to have occurrences of *S. pungens*. Subsequently, stops were made at any estuaries, river mouths, lagoons, dune complexes, salt marshes, and any other coastal feature that was likely to sustain conditions favorable to *S. pungens*. Sites were explored on foot or by canoe. If occurrences were found, GPS points were taken and if the population was larger than 3m², a full survey was done. Full surveys included walking the perimeter of the population with GPS, taking soil samples (10cm cores from 5 locations along transects), groundwater salinity (taken at low tide) and sampling vegetation. Vegetation was sampled using three randomly located 50m transects with 5 equally spaced 1m² quadrats in which percent cover of species was recorded. Morphological data was collected along the same transects within 0.25m² quadrats including

maximum height (average of 5 tallest culms), culm number, inflorescence number, leaf number per culm, and diameter at base of plant. If fertile material was present it was collected from within the 0.25m² quadrats and placed in brown paper bags and placed in coolers.

Surveys extending from the Tijuana Estuary to the Canadian border showed a surprisingly limited and patchy coastal distribution compared to historical herbarium samples, being virtually absent in brackish and salt marshes in central and southern California during the survey period (June -August 2014-2015). Historical herbarium samples showed presence of *S. pungens* along the entirety of the Pacific Coast but many previously sampled populations were not corroborated during field sampling in 2014 and 2015. *S. pungens* was observed to grow with fair predictability in the Pacific Northwest on leading edges of coastal estuaries and at river mouths, often inundated by seawater for full tidal cycles. This distribution pattern did not continue in central and southern California, where *S. pungens* retreated inwards up rivers and behind barrier dune and lagoon systems, if indeed it was present at all. From the observed patchiness and southern shift in distribution emerged a doubt regarding the true range of salt tolerance for this species. By the second year of sampling, it became apparent that *S. pungens* was virtually never found in tidal habitats that did not also receive substantial freshwater inputs, whether from rivers, streams, or groundwater. Salinity and freshwater inundation period was hypothesized to be playing a limiting role in the southern coastal distribution of *S. pungens*. Furthermore, several populations that had previously been recorded, most notably in parts of Puget Sound, appeared to have annual or cyclical variation, sometimes being absent or drastically reduced (Pers. Comm tribal biologists; Crandell, 2012) in areas that had been subject to freshwater impoundment and drought. In Lake Earl, CA, where saltwater influence is determined by barrier/breach dynamics, years with no breach resulted in dense stands of *S. pungens* compared to breach years (pers.comm.). Due to constant fluctuations in tides and freshwater inflows, it is difficult to draw more specific conclusions regarding the true range of salinity values to which field populations of *S. pungens* are regularly exposed, but populations were chosen based on the proximity to tidal influence, preference being given to populations presumed to be exposed to seawater at high tide. Field observations of Palmisano (1970), show that of the populations sampled most inhabited areas well within brackish range or roughly half that of full seawater (10-16ppt). The exception was Gray's Harbor, WA which showed much

higher salinity (24-29ppt). Skagit River, WA and Gray's Harbor, WA were included in the current study and overlap with Palmisano (1970). Additional sites from Oregon and California were included to extend the latitudinal and salinity gradient of the study.

4.2.2 Common Garden Methods:

Plants were grown from seed collected from six different sites: two in Northern California (Big Lagoon and Lake Earl), two in Oregon (Coos Bay and Necanicum River), and two in Washington (Gray's Harbor and Skagit River). Sites were chosen based on the availability of fertile material at the time of sampling, which was highly variable among populations, presumed exposure to salinity, and to cover the latitudinal distribution of populations found.

A common garden experiment was conducted at Oregon State University in Corvallis, Oregon using two concurrent randomized block designs with three factors: salinity (three levels: fresh – 0 ppt, brackish – 15ppt, and high salt - 30ppt), inundation (two levels: tidal and non-tidal), and site of origin (six levels: Big Lagoon [CA], Lake Earl [CA], Coos Bay [OR], Necanicum [OR], Gray's Harbor [WA], and Skagit River [WA]; Figure 2). Tidal and non-tidal treatments were not randomly blocked due to the constraints of plumbing, and were therefore conducted side-by-side.

Salinity treatments consisted of three levels: fresh (0ppt), brackish (15ppt), and salt (30ppt) water. Instant Ocean[®] Sea Salt mix was used to achieve the desired concentration in the brackish and salt treatments. The holding tanks for each treatment contained identical pumps (Prima XL 3400), UV sterilizer and filter (Aquatop UVFK-13), and aeration stones from a single air pump. The UV sterilizer worked very well for eliminating any algae growth in the tanks. One application of nutrient solution was applied at the beginning of each growing season. The holding tanks provided the water for both the tidal and non-tidal experiments, ensuring a single water source for each treatment. Salinity was maintained with addition of Instant Ocean[®] Sea Salt mix as needed throughout the year.

Seeds were germinated indoors during the winter of 2015/2016 (Germination Study forthcoming). Prior to germination, seeds were washed in a 1% bleach solution for 1 minute and rinsed with distilled water. Seeds were placed on DI dampened Grade 1 Whatman's filter paper disks. The disks were then placed in clear plastic petri dishes and sealed with parafilm. Germination was conducted indoors under UV growing lights with 14 hours of light at a constant temperature of 72F. Germinated seedlings were transferred individually to multiple 72-celled seedling trays and covered with a clear plastic humidity dome and placed back in the germination room under UV lights. The media used was the same pasteurized soil and Oregon beach sand mix that was used in the final pots. The seedling trays were kept consistently in 1cm of water at all times. The seedling were hardened off in the Oregon State University greenhouse for several weeks before being repotted in 15cm (6in) pots in a mix of sterilized loam and Oregon beach sand and transferred to mesocosms in spring of 2016, after the last frost. In order to avoid early mortality from transplant shock, multiple seedlings were transplanted per 6in pot and later thinned to one individual per pot. Once in the outdoor mesocosm, seedlings were given an establishment period in freshwater of one month prior to the first addition of salt.

Both the tidal and non-tidal experiments were housed next to each other outdoors in a south-facing lot protected from strong winds between two greenhouses. Shade cloth (50%) was used during midsummer as the Willamette Valley can exceed temperatures and hours of intense sun found at any of the sites of origin. The shade cloth helped control for any sun or temperature stress that could affect the results.

The non-tidal experiment contained 90 total plants in individual 6in (15 cm) diameter pots distributed between 15 clear plastic totes. Each tote was filled to six inches with water from the same tanks used for the tidal treatment. The level of water, which resulted in 5 cm of inundation of the above-ground portion of the plant, was maintained throughout the experiment by addition of water if necessary. Each tote contained 6 pots, one pot from each site of origin. Each tote was randomly assigned a salinity treatment, with 5 totes in each of the three salinity treatments. This resulted in 30 pots per salinity treatment and each site having 15 total replicates, with 5 replicates in each salinity treatment.

The 15 “tidal” mesocosms consisted of intermediate bulk containers (IBCs) which had been cut in half. These containers were filled with 6in of coarse pumice stone in order to maintain a uniform level upon which the pots would be placed, thereby ensuring that the inundation level could be adjusted to be identical between mesocosms. Inundation was adjusted to be three inches above the pot surface at “high tide” and to be 3cm above the bottom of the pot at “low tide”, for a total daily fluctuation of 24cm. This level was chosen in order to ensure that the root zone was drained during each cycle, but that soil did not dry out completely. Each IBC container was plumbed using PVC and connected to either the freshwater (0 ppt), brackish (15ppt), or salt (30ppt) water source. The initially implemented mixed semi-diurnal (6 hour cycle) automated system proved unreliable and therefore draining and filling were conducted manually during the growing season on a reduced 12 hour diurnal cycle. Water was drained and filled once daily, resulting in a “diurnal tidal cycle”. Tidal cycling was stopped during the winter months not only because of plant senescence, but to insulate plants from a hard freeze and to protect the plumbing from freezing. All tanks and containers were kept on two courses of concrete blocks and were therefore deemed to be more vulnerable to frost than they would have been in their sites of origin, where frozen sediments are extremely rare due to coastal climatic ameliorization. Precautions were taken in over-wintering the experimental set-up by putting hay bales around all containers and covering with heavy black plastic tarps. Formation of more than 2 in of ice was not observed when the set-up was checked during severe winter events, and there was no indication of cold-induced mortality at the beginning of the 2nd growth season.

Biomass was collected in September of 2017, 18 months after planting, allowing two full growing seasons in the mesocosms. All above-ground biomass was trimmed at soil height and placed in brown paper bags to be processed in drying ovens (Figure 4.1). The remaining below-ground biomass was rinsed of soil and likewise bagged and dried in ovens. Once dried to a consistent weight, above-ground and below-ground biomass were recorded for each individual. Total biomass and root to shoot ratio (RSR) were calculated from these weights.

4.2.3 Statistical Methods

Data exploration was carried out following the protocol described in Zuur, Ieno & Elphick (2010). The data were unbalanced, most notably between the inundation levels (tidal n

= 284, non-tidal n = 83, see Appendix X for experimental design). The difference in numbers between groups was due to space limitations and was not connected to death of individuals or confounding factors. Dead individuals were defined as those having either no below-ground biomass or less than 0.1 grams total biomass. Dead individuals were included in the above and below-ground analysis by adding a constant (1) to all values to allow for inclusion in the analysis. This decision was due to dead individuals showing a clear negative response to salt treatment; eliminating them would have altered the treatment response significantly. Subsequent analysis on root-to-shoot ratio, on the other hand, included only live individuals, as the allocation strategy of ‘successful’ (living at time of sampling) individuals was of interest.

Generalized linear models (GLMs) were used to evaluate the effect of the two treatments (salinity and inundation) on plant biomass (above, below, and root-to-shoot ratio), using R (R Core Team, 2018). Models were fitted using a Gamma error structure with an inverse link function, to allow for the relaxation of the homoscedasticity assumption and non-linear response. To model RSR, a Poisson GLM with log link function was used. Fixed covariates were site of origin (categorical with 6 levels: Big Lagoon, CA, Lake Earl, CA, Coos Bay, OR, Necanicum River, OR, Gray’s Harbor, WA, and Skagit River, WA), salinity (categorical with 3 levels: fresh 0ppt, brackish 15ppt, and salt 30ppt), and inundation (categorical with two levels: tidal with daily drying period and non-tidal with consistent inundation). Three model sets (one for each response variable) were constructed, each comprising all covariate combinations above-ground. AIC and ANOVA were used to evaluate inclusion of model terms and ranked to find the most parsimonious model. Model assumptions were verified by plotting Pearson residuals versus fitted values, versus each covariate in the model. Model residuals, Pearson’s coefficients, residual deviance, and Cook’s distance were calculated.

Estimated marginal means and subsequent pairwise comparisons with Bonferroni adjustment were attained using the package ‘emmeans’ (Lenth, 2021) in software R. Visualization of results was conducted using the package ‘ggpot2’ (Wickham, 2009). Results and discussion are based on estimated marginal means calculated based on the reference grids produced using the GLMs for each response variable. All comparisons were conducted using the emmeans package in R software and were computed using the estimate marginal means. This was done to account for unbalanced data.

4.3 RESULTS

Salinity, site of origin and inundation all influenced final above and below ground biomass. Salinity and inundation influenced allocation of biomass (RSR). Additionally, the relationship between RSR, above, below, and total biomass and salinity differed with inundation. The relationship between above-ground biomass and salinity also differed by site of origin. In all cases, adding the salinity x inundation term improved AIC and decreased deviance.

Table 4.1: GLM model terms

A.	Above ground biomass					
	Df	Deviance	Resid. Df	Resid. Dev	Pr(>Chi)	
Null			373	59.17		
salinity	2	5.45	371	53.72	3.40E-13	*
inundation	1	8.16	370	45.57	1.86E-20	*
site	5	3.86	365	41.7	1.07E-07	*
salinity:inundation	2	1.02	363	40.69	0.004689	*
salinity:site	10	3.8	353	36.88	1.66E-05	*
B.	Below ground biomass					
	Df	Deviance	Resid. Df	Resid. Dev	Pr(>Chi)	
Null			373	143.81		
salinity	2	24.1	371	119.71	4.03E-27	*
inundation	1	0.1	370	119.61	0.478562	
site	5	5.71	365	113.9	2.52E-05	*
salinity:inundation	2	23.02	363	90.88	6.15E-26	*
C.	Total biomass					
	Df	Deviance	Resid. Df	Resid. Dev	Pr(>Chi)	
Null			373	100.33		
salinity	2	17.27	371	83.06	1.61E-26	*
inundation	1	0.17	370	82.89	0.274374	
site	5	5.17	365	77.72	1.15E-06	*
salinity:inundation	2	13.29	363	64.42	1.38E-20	*
D.	Root-to-shoot ratio (RSR)					

	Df	Deviance	Resid. Df	Resid. Dev	Pr(>Chi)	
Null			373	401.37		
salinity	2	31.58	371	369.79	1.39E-07	*
inundation	1	62.48	370	307.31	2.69E-15	*
salinity:inundation	2	66.11	363	236.97	4.41E-15	*

The addition of a second interaction term of salinity* site reduced deviance and lowered AIC for above-ground biomass. Removing the site term reduced deviance and lowered AIC in the model for root-to-shoot ratio. Residuals showed no abnormal patterning. The models were not found to be overdispersed. All dead individuals were in the tidal treatment and all except one were in the salt treatment. Eight out of the eleven dead came from the Coos Bay source population. The single dead individual from the brackish tidal treatment was from the Coos Bay source population.

Table 4.2: For all response variables (above, below, total, and root-to-shoot ratio) the model with lowest AIC was selected, resulting in the following models:

GLM	AIC
Above-ground biomass ~ salinity + inundation + site + <u>salinity:site</u> + <u>salinity:inundation</u>	1157.69
Below-ground biomass ~ salinity + inundation + site + <u>salinity:inundation</u>	2229.05
Total mass ~ salinity + inundation + site + <u>salinity:inundation</u>	2352.67
Root-to-shoot ratio ~ salinity + inundation + <u>salinity:inundation</u>	1256.01

Table 4.3.a: Estimated marginal means and raw means of above-, below-, and total biomass of *S. pungens* for the non-tidal condition calculated for each combination of salinity treatment and site of origin.

		Non-tidal									
			Above				Below				
Salinity	Site	Count	Raw Mean	SE	EMM	SE	Raw Mean	SE	EMM	SE	Raw Mean
Fresh	Big Lagoon, CA	5	3.21	0.59	2.85	0.21	10.64	1.68	11.03	1.07	13.85
	Lake Earl, CA	5	3.02	0.21	2.93	0.22	13.20	2.97	12.12	1.25	16.22
	Coos Bay, OR	5	1.83	0.55	2.45	0.17	4.69	2.32	8.69	0.73	6.52
	Necanicum River, OR	5	3.07	0.19	2.99	0.22	13.04	3.20	11.56	1.13	16.11
	Gray's Harbor, WA	5	2.71	0.26	2.70	0.19	11.94	2.90	11.18	1.08	14.65
	Skagit River, WA	5	2.95	0.30	2.87	0.26	10.05	1.97	8.98	0.88	13.01
Brackish	Big Lagoon, CA	5	2.66	0.12	2.84	0.20	7.29	0.99	10.71	1.03	9.95
	Lake Earl, CA	5	2.48	0.18	2.79	0.20	12.32	4.38	11.73	1.20	14.80
	Coos Bay, OR	5	2.76	0.36	2.66	0.18	8.85	1.52	8.48	0.71	11.61
	Necanicum River, OR	5	3.16	0.26	2.87	0.20	10.46	1.73	11.21	1.08	13.62
	Gray's Harbor, WA	5	2.72	0.23	2.80	0.19	11.69	2.18	10.85	1.04	14.41
	Skagit River, WA	5	2.49	0.70	2.32	0.20	11.14	3.48	8.77	0.85	13.63
Salt	Big Lagoon, CA	5	2.63	0.21	2.82	0.23	10.47	0.84	14.12	1.48	13.10
	Lake Earl, CA	5	2.90	0.27	3.11	0.26	18.05	3.78	15.94	1.80	20.95
	Coos Bay, OR	5	2.26	0.33	1.74	0.13	13.95	3.24	10.49	0.94	16.21
	Necanicum River, OR	5	3.34	0.31	3.33	0.27	12.43	2.68	15.00	1.56	15.77
	Gray's Harbor, WA	5	2.72	0.21	2.98	0.24	17.49	2.02	14.36	1.48	20.21
	Skagit River, WA	5	2.79	0.49	2.66	0.26	8.44	0.98	10.92	1.19	11.23

Table 4.3.b: : Estimated marginal means and raw means of above-, below-, and total biomass of *S. pungens* for the tidal condition calculated for each combination of salinity treatment and site of origin.

		Tidal										
		Above					Below				Total	
Salinity	Site	Count	Raw Mean	SE	EMM	SE	Raw Mean	SE	EMM	SE	Raw Mean	SE
Fresh	Big Lagoon, CA	15	3.95	0.15	4.07	0.30	16.67	1.04	14.89	1.20	20.62	1.12
	Lake Earl, CA	15	4.21	0.23	4.24	0.32	14.80	1.16	16.93	1.42	19.01	1.36
	Coos Bay, OR	15	3.52	0.17	3.32	0.25	12.98	0.72	10.91	0.82	16.51	0.84
	Necanicum River, OR	25	4.36	0.19	4.38	0.26	15.86	0.89	15.87	1.10	20.22	1.02
	Gray's Harbor, WA	20	3.77	0.18	3.78	0.25	13.66	1.24	15.16	1.12	17.43	1.33
	Skagit River, WA	5	4.04	0.79	4.12	0.48	12.35	2.30	11.38	1.19	16.39	3.08
Brackish	Big Lagoon, CA	15	4.89	0.29	4.83	0.37	13.34	0.68	12.89	0.95	18.22	0.88
	Lake Earl, CA	15	4.77	0.34	4.67	0.35	14.25	0.67	14.40	1.09	19.02	0.87
	Coos Bay, OR	15	4.29	0.45	4.32	0.33	10.77	1.14	9.80	0.68	15.06	1.42
	Necanicum River, OR	25	4.85	0.27	4.91	0.29	12.91	0.43	13.62	0.88	17.76	0.52
	Gray's Harbor, WA	19	4.73	0.26	4.71	0.32	13.55	0.83	13.10	0.90	18.28	1.01
	Skagit River, WA	5	3.31	0.71	3.49	0.41	8.21	1.61	10.17	0.97	11.52	2.32
Salt	Big Lagoon, CA	15	3.23	0.46	3.16	0.23	5.92	0.97	5.66	0.30	9.15	1.38
	Lake Earl, CA	15	3.60	0.34	3.52	0.26	6.95	1.03	5.93	0.32	10.55	1.31
	Coos Bay, OR	15	1.69	0.21	1.86	0.13	1.97	0.35	4.97	0.25	3.66	0.54
	Necanicum River, OR	25	3.81	0.16	3.81	0.22	6.89	0.61	5.79	0.29	10.70	0.69
	Gray's Harbor, WA	20	3.43	0.26	3.36	0.22	5.59	0.61	5.70	0.29	9.02	0.84
	Skagit River, WA	5	2.83	0.54	2.96	0.32	5.10	1.33	5.06	0.30	7.93	1.85

4.3.1 Above-ground biomass

The estimated marginal means of above-ground biomass in the non-tidal treatment ranged from a high of $3.33 \text{ g} \pm 0.27 \text{ SE}$ in Necanicum River, OR to the lowest of $1.74 \text{ g} \pm 0.13 \text{ SE}$ for Coos Bay, OR; with both extremes found in the salt treatment (Table 4.3.a). The range in the tidal treatment was from $4.91 \text{ g} \pm 0.29 \text{ SE}$ for Necanicum River, OR in the brackish treatment to $1.86 \text{ g} \pm 0.13 \text{ SE}$ for Coos Bay, OR in the salt treatment (Table 4.3.b).

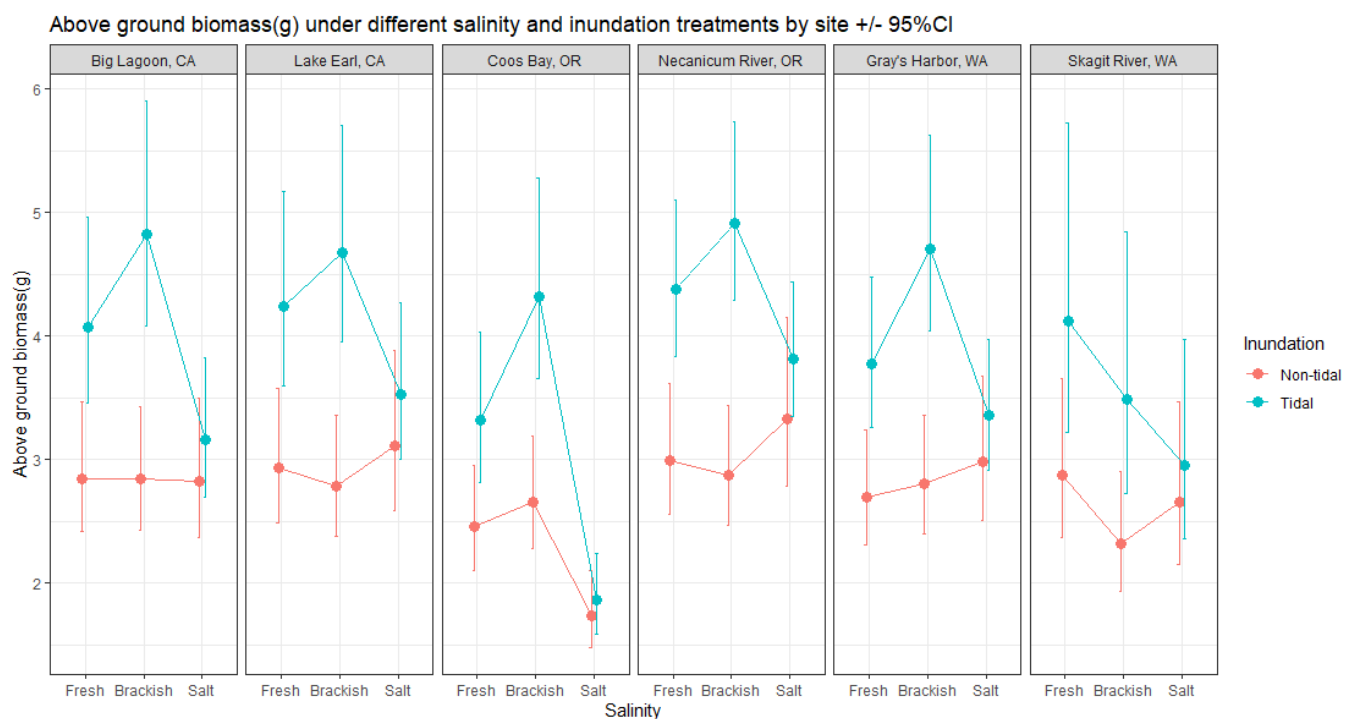


Figure 4.2: Estimated marginal means $\pm 95\%$ CI of above-ground biomass under different salinity and inundation treatments grouped by site of origin.

Pairwise comparisons between marginal means of above-ground biomass within sites showed no difference between salinity treatments in the non-tidal condition, save in Coos Bay, OR (Appendix IV: Table IV.1, $p > 0.5$ for all comparisons. Figure 4.2, (red points)). The exception was Coos Bay, OR which showed between 0.21 and 1.23 g more mean above-ground biomass in the fresh treatment than in the salt treatment in the non-tidal condition (95% CI, $p = 0.002$) and between 0.39 and 1.46 g more in the brackish than the salt treatment (95%CI, $p = 0.0001$). In the tidal condition, all sites save Skagit River, WA showed significantly more mean

above-ground biomass in brackish water compared to salt, with Coos Bay, OR showing the largest difference of 1.62 to 3.30g 95% CI ($p < 0.001$) more in brackish water (Figure 4.2, (blue points)). Skagit River, WA showed no difference in mean above-ground biomass between salinity treatments in either inundation condition ($p > 0.1$). Big Lagoon, CA and Coos Bay, OR showed more mean above-ground biomass in the fresh treatment compared to salt. No difference was found between fresh and brackish treatments regardless of inundation condition (Appendix IV: Table IV.2, $p > 0.05$).

Pairwise comparisons of marginal means of above-ground biomass between inundation treatments within sites showed that fresh and brackish water treatments had lower mean above-ground biomass in the non-tidal treatment compared to tidal (Appendix IV: Table IV.3, $p < 0.05$ for all comparisons, Figure 4.2, comparison of blue and red points). In all sites except Skagit River, WA, these differences between inundation were even larger within the brackish treatment. In other words, regardless of site of origin, plants in fresh and brackish water with daily drying (tidal treatment) showed higher mean above-ground biomass than those consistently inundated (non-tidal treatment), with the difference between the inundation treatments being larger in the brackish treatment. Interestingly, this difference in above-ground biomass between inundation regimes was not significant in the salt treatment ($p > 0.05$ for all comparisons). Those plants exposed to salt water showed no significant difference in marginal means between tidal and non-tidal treatments.

Comparisons of estimated marginal means of above-ground biomass between sites accounting for inundation and salinity treatment showed that plants from Coos Bay, OR produced less above-ground biomass than other sites within the salt treatment (Figure 4.2). This is most likely due to the high mortality of Coos Bay, OR individuals. Plants from the Coos Bay, OR source population produced lower mean above-ground biomass under salt conditions in both tidal and non-tidal treatments compared to all other sites, ranging from the smallest difference of 0.41 to 1.76 g 95% CI ($p < 0.001$) less than Big Lagoon, CA (tidal treatment) to the largest difference of 1.2 to 2.7g 95% CI ($p < 0.001$) less than Necanicum River, OR (non-tidal). Differences were larger between Coos Bay, OR and all other sites in the non-tidal versus tidal treatment. Necanicum River, OR produced more mean above-ground biomass than Coos Bay, OR in the fresh x tidal treatment ($p = 0.04$).

4.2.2 Below-ground biomass results

The estimated marginal means of below-ground biomass in the non-tidal treatment ranged from $15.94 \text{ g} \pm 1.80 \text{ SE}$ in Lake Earl, CA in the salt treatment to $8.48 \text{ g} \pm 0.71 \text{ SE}$ for Coos Bay, OR in the brackish treatment (Table 4.3.a). The range in the tidal treatment was from $16.93 \text{ g} \pm 1.42 \text{ SE}$ for Lake Earl, CA in the fresh treatment to $4.97 \text{ g} \pm 0.25 \text{ SE}$ for Coos Bay, OR in the salt treatment (Table 4.3.b).

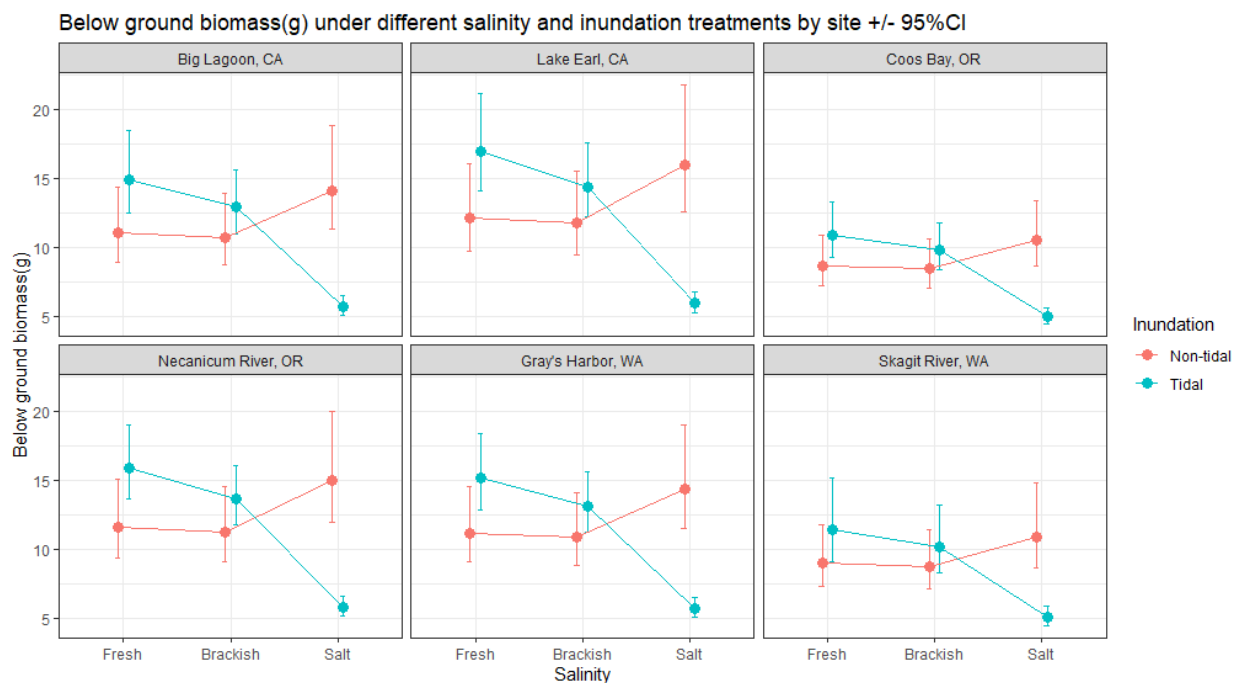


Figure 4.3: Estimated marginal means $\pm$ 95 % CI of below-ground biomass under different salinity and inundation treatments grouped by site of origin.

Pairwise comparisons between marginal means of below-ground biomass within sites showed no difference between salinity treatments in the non-tidal treatment (Appendix IV: Table IV.1, $p > 0.05$). Across all sites, plants in the salt treatment produced significantly less mean below-ground biomass than in either the brackish or fresh treatments in the tidal inundation treatment. The largest difference in mean below-ground biomass was seen in the Lake Earl, CA source population with between 7.8 and 14.21g more mean below-ground biomass in the fresh

treatment compared with salt (95% CI, $p < 0.001$). Differences were consistently larger between fresh and salt than between brackish and salt. No difference was found in mean below-ground biomass between fresh and brackish treatments regardless of inundation treatment (Appendix IV: Table 2, $p > 0.05$ for all comparisons).

Pairwise comparisons between marginal means of below-ground biomass of tidal and non-tidal treatments within sites showed lower mean below-ground biomass in the non-tidal than tidal treatment in both the fresh and brackish treatments (Appendix IV: Table IV.3, $p < 0.05$ for all comparisons except Skagit River, WA in the brackish treatment, with $p = 0.07$). The differences were larger between inundation treatments in the fresh treatment, for all sites. Interestingly, this trend reversed in the salt treatment which had significantly more mean below-ground biomass in the non-tidal treatment than in the tidal treatment (Appendix IV: Table IV.3, $p < 0.05$ for all comparisons). In other words, in the high salt treatment those plants with consistent inundation (non-tidal) produced more mean below-ground biomass than those exposed to daily drying (tidal).

Mean below-ground biomass for Coos Bay, OR was lower than Lake Earl, CA, Necanicum River, OR, and Gray's Harbor, WA across all salinity treatments and inundation treatments (Appendix IV: Table IV.1&2, $p < 0.05$ for all pairwise comparisons). Additionally, Coos Bay, OR had less mean below-ground biomass than Big Lagoon, CA in all salinity treatments in the tidal treatment (Appendix IV, : Table IV.1&2, $p < 0.05$ for all pairwise comparisons). Skagit River, WA produced less mean below-ground biomass than Lake Earl, CA and Necanicum River, OR in the tidal treatment in fresh and brackish treatments (Appendix IV, : Table IV.1&2, $p < 0.05$ for all pairwise comparisons). Skagit River, WA produced less mean below-ground biomass than Lake Earl, CA in the salt treatment in both tidal and non-tidal treatments (Appendix IV: Table IV. 1&2 $p < 0.05$ for all pairwise comparisons).

4.2.3 Total biomass results

The estimated marginal means of total biomass in the non-tidal treatment ranged from $18.64 \text{ g} \pm 1.72 \text{ SE}$ in Lake Earl, CA in the salt treatment to $10.83 \text{ g} \pm 0.77 \text{ SE}$ for Coos Bay, OR in the brackish treatment (Table 4.3.a). The range in the tidal treatment was from $21.07 \text{ g} \pm 1.47$

SE for Lake Earl, CA in the fresh treatment to $7.66\text{g} \pm 0.34$ SE for Coos Bay, OR in the salt treatment (Table 4.3.b).

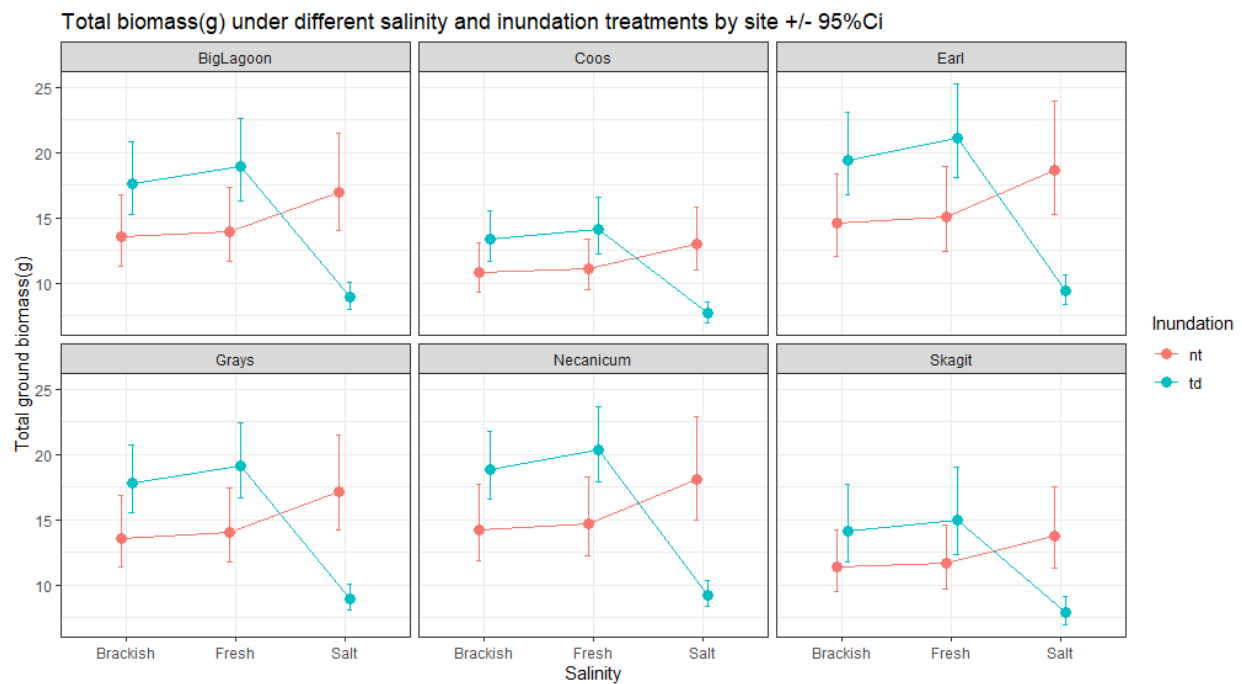


Figure 4.4: Estimated marginal means $\pm$ 95 % CI of total biomass under different salinity and inundation treatments grouped by site of origin.

Comparisons of marginal means of total biomass when grouped by inundation and salinity treatment showed no difference between salinity treatments in the non-tidal treatment (Figure 4.4, Appendix IV: Table IV.2 & Table IV.1, $p > 0.05$ all comparisons). For all sites, mean total biomass in the tidal treatment was lower in the salt water treatment than in either the fresh or brackish treatments (Appendix IV: Table IV.2, $p < 0.05$ for all comparisons). The largest difference in mean total biomass was seen in the Coos Bay, OR source population with between 8.47 and 14.96g more mean total biomass in the brackish treatment than in the salt (95% CI, $p < 0.001$). The differences were largest between brackish and salt treatments rather than between fresh and salt, as seen in the below-ground biomass. Once again, no difference was found in mean biomass between fresh and brackish treatments regardless of inundation condition or salinity treatment (Appendix IV: Table IV.1, $p > 0.05$).

Pairwise comparisons between marginal means of total biomass between inundation treatments within sites showed lower mean total biomass in the non-tidal treatment compared to tidal in both the fresh and brackish treatments (Appendix IV: Table IV.2, $p < 0.05$ for all comparisons). This trend reversed in the salt treatment which had significantly more mean total biomass in the non-tidal treatment compared to the tidal treatment (Appendix IV: Table IV.2, $p < 0.05$ for all comparisons).

Mean total biomass for Coos Bay, OR was lower than Big Lagoon, CA, Lake Earl, CA, Necanicum River, OR, and Gray's Harbor, WA across all salinity treatments and inundation treatments (Appendix IV: Table IV.3, $p < 0.05$ for all pairwise comparisons). Skagit River, WA produced less mean total biomass than Lake Earl, CA and Necanicum River, OR across all inundation and salinity treatments (Appendix IV: Table IV.3, $p < 0.05$ for all pairwise comparisons).

4.2.4 RSR results

The model selection process for root-to-shoot ratio indicated that site was not a significant factor and was therefore not included in the GLM. Therefore, pairwise comparisons do not include site. RSR gives a measure of the similarity between the value of the below-ground biomass versus the above-ground biomass. RSR speaks only to the allocation strategy and does not give any information regarding the total amount of biomass. If the ratio approaches 1, it means that the below and above-ground values are approaching each other (an equal amount above and below). If the ratio were to be above 1, this means there is more below-ground biomass than above-ground biomass. If the ratio is less than one, there is more above-ground biomass than below-ground biomass. All RSR scores were over one, reflecting the dominance of below-ground biomass production in *S. pungens* (Table 4.4) and can be interpreted as how many times more biomass was produced below vs. above. Both the highest (non-tidal $RSR = 4.73 \pm 0.40$) and the lowest (tidal $RSR = 1.23 \pm 0.11$) estimated marginal mean RSR were in the salt treatment but in opposite inundation treatments (Table 4.4).

Table 4.4: Estimated marginal means (EMM) and standard error (SE) for root-to-shoot ratio

	Non-tidal		Tidal	
Salinity	EMM	SE	EMM	SE
Fresh	3.20	0.33	3.23	0.18
Brackish	3.70	0.35	2.30	0.16
Salt	4.73	0.40	1.23	0.11

(RSR) of *S. pungens* calculated for each combination of tidal condition and salinity treatment.

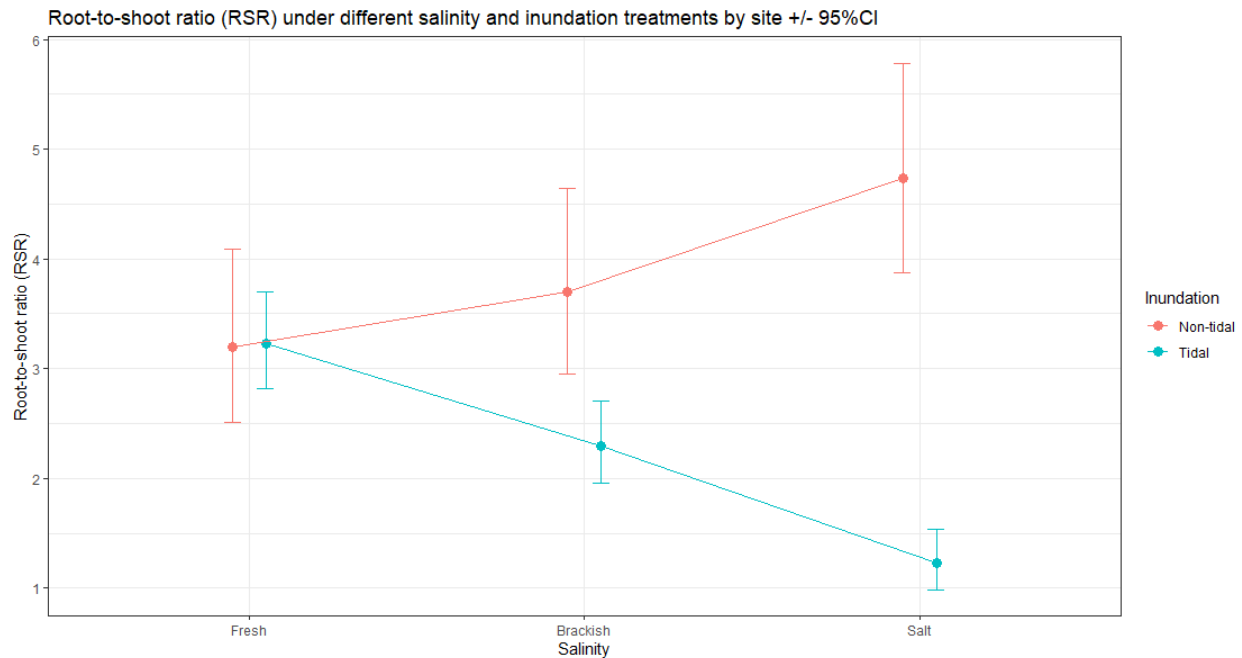


Figure 4.5: Estimated marginal means $\pm$ 95 % CI of RSR under different salinity and inundation treatments.

Comparisons within the tidal treatment showed significant difference in mean RSR between all salinity treatments (Figure 4.5 and Appendix IV: Table IV.4). RSR in fresh water ($RSR = 3.23 \pm 0.18$ SE) was significantly higher than in both brackish ($RSR = 2.3 \pm 0.16$ SE) and salt water ($RSR = 1.23 \pm 0.11$ SE), and higher in brackish than in salt (Appendix IV: Table IV.4, all pairwise comparisons with Bonferroni adjustment $p < 0.05$).

In the non-tidal treatment, mean RSR was found to be between 0.3 and 2.76g (95%CI, $p = 0.008$) higher in the salt treatment than in the freshwater treatment (Appendix IV: Table IV.4). Interestingly, within the non-tidal inundation treatment, mean RSR did not differ significantly between fresh and brackish or between brackish and salt treatments (Appendix IV: Table IV.4, $p > 0.05$ for both comparisons; Figure 4.5: red points). Remembering that mean total biomass was not found to be significantly different between salinity treatments in the non-tidal treatment, the difference in RSR shows a shift in allocation below-ground between fresh and salt treatments. Therefore, although no statistical differences were found in the above, below, and total biomass between salinity treatments in the non-tidal treatment, RSR shows a significant increase in the proportion of biomass allocated below-ground under high salt conditions ($RSR = 4.73 \pm 0.4$ SE) compared with fresh water ($RSR = 3.2 \pm 0.33$ SE) (Appendix IV: Table IV.4, pairwise comparison with Bonferroni adjustment $p = 0.008$).

Within salinity treatments, estimated marginal means for RSR showed differences between inundation treatments for brackish and fresh treatments. Estimated marginal means were lower in the tidal treatment than the non-tidal treatment for both brackish (between 0.65 and 2.16 lower RSR, $p < 0.001$) and salt treatments (between 2.69 and 4.31 lower RSR, $p < 0.001$). Mean RSR did not differ between inundation treatments in fresh water (Appendix IV: Table IV.4, $p > 0.05$).

4.4 DISCUSSION

4.4.1 Biomass production

My results suggest that *S. pungens* performs best in freshwater conditions but can tolerate saline conditions, though the degree of tolerance differed dramatically between inundation conditions. Increasing salinity had no detrimental effect on biomass production in both tidal and non-tidal treatments across nearly all the study populations. Only plants from Coos Bay, OR had higher total biomass in fresh than in brackish condition. No previous studies have investigated the effect of salinity on biomass production in *S. pungens*. Studies that have investigated other species in the genus have found that while tolerance to salinity is highly species specific, most species show some detrimental effects of increased salinity (Batool et al. 2013; Howard 2010). Howard (2010) found that even moderate levels of salinity reduced growth of *S. californicus* and

S. robustus, whereas Batool et al. (2013) found variable response for *Schoenoplectus triqueter*, *S. lacustris*, and *S. juncooides* at lower salinities but an overall reduction in growth at higher salinities. *Schoenoplectus triqueter* shoot and root dry biomass actually increased at low salinity but decreased at higher salinity (Batool et al. 2013). Root dry biomass of *S. juncooides* increased with increasing salinity (Batool et al. 2013).

Tidal inundation and salinity interacted to influence biomass production. Tidal inundation generally increased biomass production under fresh and brackish conditions. A study conducted on closely related species, *Schoenoplectus acutus* and *Schoenoplectus americanus*, showed more reduced biomass with increasing inundation for *S. acutus* than for *S. americanus*, tracking with other studies of *S. americanus* under abiotic stress (Broome et al., 1995; Kirwan & Guntenspergen, 2012; Seliskar, 1990). Visser and Peterson (2015) found that below-ground biomass of *Spartina patens* increased with flooding under fresh conditions but decreased under brackish conditions and found reduced growth when soil was inundated. Janousek et al. (2016) found parabolic relationships between inundation and productivity of *Spartina foliosa*, *Bolboschoenus maritimum*, and *Carex lyngbyei* with maximum total plant biomass at 38, 28, and 15% submergence respectively, while *Salicornia pacifica* and *Juncus balticus* decreased biomass with increasing inundation. RSR varied inconsistently between species, suggesting the need to investigate species-level responses in marsh plants to inundation (Janousek et al. 2016).

Comparisons within inundation treatments between salinity treatments showed differing impacts of salinity on mean biomass production. In both fresh and brackish treatments, tidal treatment resulted in higher mean above, below, and total biomass than non-tidal treatment. Under the high salt treatment, consistent inundation (non-tidal treatment) resulted in higher below-ground and total biomass than those plants exposed to daily drying (tidal treatment), while above-ground biomass showed no difference between inundation treatments. Janousek and Mayo (2013) found a disproportionate reduction in below-ground biomass, similar to our results. The lack of difference in above-ground biomass in the non-tidal treatment may have been due the formation of aerenchyma in response to decreased oxygen availability, which in turn increased internal airspaces and reduced overall tissue biomass. Howard (2010) found above and below-ground biomass of *Distichlis spicata* to be lower in tidal compared to saturated (non-tidal) conditions, although *Juncus roemerianus* was not affected.

The effect of salinity on mean biomass production differed between inundation treatments, suggesting a strong relationship between daily drying and degree of tolerance of *S. pungens* to high salt concentrations. In the tidal treatment, plants in the high salt treatment produced significantly less mean below-ground (38-44%) and total biomass (46-51%) than in either the brackish or fresh treatments. No difference in above, below, or total biomass was found between the three salinity treatments in the non-tidal treatment (excluding Coos Bay). These results suggest that either a) consistent access to water in the non-tidal treatment had a mitigating effect on the osmotic stress or b) daily drying down in the tidal treatment exacerbated negative impact of salinity on *S. pungens* biomass production. It has been suggested that species with low salt tolerance may be more susceptible to interactive effects, since extended flooding both prolongs exposure to osmotic stress and leads to salt accumulation in the soil (Janousek and Mayo, 2013; Spalding and Hester, 2007). *S. pungens* survival under both tidal and non-tidal flooding leads to the conclusion that *S. pungens* may have a high degree of salt tolerance.

The negative interaction between daily drying in the tidal treatment and high salinity for *S. pungens* is contrary to results found in other studies focused on different marsh species, in which biomass productivity was reduced when paired with increased inundation. Elevated salinity has been observed to result in a marked reduction of primary productivity (McKee and Mendelssohn 1989; Gough and Grace 1998; Willis and Hester 2004), with many marsh species showing reduction of below-ground biomass under the combined stressors of increased salinity and inundation (Stagg et al. 2013). Many studies have looked at marsh species response to both salinity and inundation: *Carex lyngbyei*, *Triglochin maritima*, and *Argentina pacifica* (Buffington et al., 2020); *Distichlis spicata*, *Juncus roemerianus*, and *Spartina bakeri* (Howard et al. 2016); *Distichlis spicata*, *Phragmites australis*, *Schoenoplectus californicus*, and *Schoenoplectus robustus* (Howard, 2010); *Achillea millefolium*, *Agrostis stolonifera*, *Deschampsia cespitosa*, *Grindelia stricta*, *Juncus balticus ssp. ater*, *Plantago maritima*, and *Triglochin maritima* (Janousek and Mayo, 2013); *Juncus roemerianus* and *Spartina alterniflora* (Pennings et al, 2005); *Panicum hemitomon*, *Sagittaria lancifolia*, and *Spartina patens* (Spalding and Hester, 2007; Visser and Peterson, 2015), *Sarcocornia pacifica* (Schile et al. 2011; Woo and Takekawa, 2012). Konisky and Burdick (2004) and Spalding and Hester (2007) found negative interactive effects of inundation duration and salinity on primary production of the marsh species

studied. Howard (2010) found that consistent inundation (non-tidal treatment) paired with high salinity had negative impacts on growth for *Schoenoplectus californicus*. Janousek and Mayo (2013) found increased flooding to reduce shoot and root growth in all species studied (*Achillea millefolium*, *Agrostis stolonifera*, *Deschampsia cespitosa*, *Grindellia stricta*, *Juncus balticus* ssp. *Ater*, *Plantago maritima*, and *Triglochin maritima*). Schile et al. (2011) found that salinity had a negative impact on biomass of *Sarcocornia pacifica* in poorly-drained areas compared to well-drained areas. Visser and Peterson (2015) found *Sagittaria lancifolia* growth and biomass to be reduced by both increased salinity and flood duration. One study found a similar trend to those found in this study for *Distichlis spicata*, with above and below-ground biomass lower in tidal compared to saturated conditions (Howard, 2010). Several studies on a closely related species, *S. americanus*, suggest that it occupies a lower elevation within the marsh and is more tolerant of stress (Broome et al., 1995; Kirwan & Guntenspergen, 2012; Seliskar, 1990).

Although not the focus of this study, observations of unexpected differences in root morphology between inundation treatments may help to explain differences in mean below-ground biomass between tidal and non-tidal treatments. It was observed that the structure of the roots in the non-tidal treatment were significantly more fibrous, consisting of a mat of fine roots as opposed to the thicker but less dense rhizomatous roots observed in the tidal treatment. This difference in morphology may have been due to decreased oxygen availability in the non-tidal treatment necessitating increases surface area for gas exchange and resulted in proliferation of fine roots.

The morphological differences observed below-ground between inundation treatments may also be responsible for the increased tolerance to high salt seen in the non-tidal treatment. The formation of robust rhizomes in the tidal treatment may be a result of thicker rhizomes being more tolerant to prolonged periods of drying, limiting water loss and tissue damage. On the other hand, dense fibrous roots which increase surface area help mitigate decreased oxygen availability but can also help with increased water uptake. Therefore, fine fibrous roots which proliferate in the consistent inundation due to decreased oxygen availability (but are too delicate for daily drying) allowed plants to offset salt stress in the high salt conditions by increasing surface area available for ion exchange. This fact, paired with the lack of significant difference in above-ground biomass, may indicate that *S. pungens* has exclusionary mechanisms or relatively

high tolerance to direct toxicity, as high salt did not result in tissue death (which would have resulted in a difference in biomass).

4.4.1 Site of origin

The response to salinity and inundation was found to be influenced by site of origin, with certain sites being more negatively impacted by salinity and drying stress, specifically Coos Bay, OR. Pennings et al. (2005) suggest that the nature of ecological interactions is likely to vary geographically because of variation in the physical environment, underlining the importance of including multiple populations in studies focused on vegetative response to abiotic stressors in order to successfully generalize the results of field studies across geographical scales. Salinity stress, for example, may play a more important role in mediating plant zonation patterns at lower latitudes (Pennings et al. 2005). Certain sites showed more vigor, producing more biomass in the fresh and brackish treatments, specifically Lake Earl, CA and Necanicum River, OR compared with Skagit River, WA and Coos Bay, OR. Coos Bay, OR produced significantly less below-ground and total biomass than most other sites regardless of inundation and salinity, suggesting overall lower vigor in the common garden growing conditions. No mortality was found in the brackish or fresh treatments therefore lower biomass cannot be attributed to simple mortality. Above-ground biomass for Coos Bay, OR was only significantly lower in the salt treatments, wherein mortality most likely accounts for the lower above-ground biomass. These results suggest that although below-ground biomass was lower than several sites in non-tidal and tidal brackish and fresh treatments, this difference in below-ground biomass did not translate to differences above-ground. Skagit River (WA) did not suffer from similar mortality, but did not produce more below-ground or total biomass than Coos Bay (OR). Skagit River, WA produced less mean total biomass than Lake Earl, CA and Necanicum River, OR across all inundation and salinity treatments. Skagit River, OR produced less mean below-ground biomass than Lake Earl, CA and Necanicum River, OR but only in the tidal treatment, suggesting that Lake Earl, CA and Necanicum River, OR may have higher tolerance to drying than Skagit River, WA.

4.4.3 Biomass allocation

S. pungens produced more below-ground biomass than above-ground biomass across all salinity, inundation, and sites of origin, suggesting that it follows the established pattern of

biomass allocation of most grasses, rushes, and sedges. A recent meta-analysis based on the global data conducted by Qi et al. (2019) found root to shoot ratio (RSR) to range from a low of 0.0001 in tropical rainforests to a high of 45.615 in cool temperate grasslands, with a global mean of 0.900 (n=7763) across all biome types. It is to be noted that any mention or inclusion of emergent or aquatic biomes or vegetation types is conspicuously missing. All RSRs for *S. pungens* were above 1, indicating that more biomass, often 3 to 4 times more, is allocated below-ground. RSR seemed to be driven in large part by changes in below-ground biomass compared to the relatively unchanging amount of above-ground biomass. This dynamic may suggest that there is a minimum amount of photosynthetic material that must be present above-ground for plant survival. This underlines the importance of assessing below-ground biomass, especially in habitats that are highly studied for shoreline stabilization and carbon sequestration, as above-ground biomass does not seem to be an adequate indicator of total biomass. An understanding of below-ground response is critical as many indices of wetland health are rarely correlated with above-ground biomass (Nyman et al. 1994; Turner et al. 2004). Root zone processes have been found to dominate the response of many marshes to sea level rise, especially where mineral sediment is limited (Turner, Swenson & Milan 2000; Cahoon et al. 2006; Nyman et al. 2006).

Results showed that inundation and salinity significantly affect the biomass allocation strategy of *S. pungens*, while site of origin did not. In high salt, RSR showed starkly diverging biomass allocation strategies between tidal and non-tidal inundation. Under consistent inundation (non-tidal), increased salinity resulted in proportionally more biomass allocated below-ground than above. This may be due to the proliferation of fibrous roots, in response to both decreased oxygen availability and water stress due to high salinity. Conversely, RSR decreased as salinity increased within the tidal treatment. Those plants exposed to daily drying of the tidal treatment allocated a larger proportion of total biomass above-ground as salinity increased. In this case, estimated marginal means show this was due to a drastic reduction in below-ground biomass as above-ground biomass remained largely unchanged. The combination of high salt and daily drying resulted in a halving of below-ground biomass, resulting in the lowest RSR. Understanding biomass allocation under inundated conditions is particularly important in the context of vertical sediment accretion and sea-level rise. Increased root biomass under inundated conditions tracks with results from Nyman et al. (2006) in which inundated marsh vegetation produced root growth above the marsh surface. Further, Nyman et al. (2006) found that vertical

accretion varied with organic accumulation rather than mineral sedimentation in a wide range of conditions in coastal Louisiana.

4.5 CONCLUSION

Although naturally-occurring saline habitats are not uncommon, a relatively small number of species can thrive in saline conditions; many could be argued to exist despite saline conditions. Understanding the impacts of changing salinity and hydrology on the dynamics governing distribution, establishment, and resilience of coastal wetland species is crucial to their continued survival and management. Results showed that under consistent inundation, *S. pungens* can withstand near seawater salinity concentrations. However, when high salinity is paired with daily drying *S. pungens* biomass production, and presumably long-term survival, suffer. No mortality was seen during the first growing season, therefore all mortality of the Coos Bay, OR population occurred during the second growing season. It is unknown whether plants in the tidal high salt treatment would have persisted for another growing season, but it was clear based on the observations following the first year that growth in the second year was reduced. It is possible that other plants would have succumbed in following years if below-ground biomass proved insufficient to overwinter. -These conclusions have important management implications as stands of *S. pungens* may benefit from increased freshwater or carefully timed plantings in areas foreseen to have salinity changes. Prolonged or repeated periods of drought or extreme tidal or storm events may lead to extensive die-back, habitat shifts, and increased shoreline erosion. The pattern observed may suggest that *S. pungens* may prefer either freshwater locations along the coast, or locations close to the sea margin with freshwater inputs, but not in intermediate sites that are subject to inundation part of the day, were repeated drying may lead to more saline conditions over time. This salinity pattern is observed in salt marshes, where (mid-marsh habitats in salt marsh are more saline than either ends of the range (compared with the water's edge or upslope margin). Furthermore, these preferences underline the importance of understanding freshwater inputs, freshwater timing, and groundwater salinity and the role they may play in vegetation distribution in salt marshes.

The results of the biomass allocation analysis suggest that below-ground biomass is disproportionately important to *S. pungens*. The most widespread and feasible methods currently

used for answering a myriad of ecological questions concerning vegetation center on methods of assessing above-ground vegetative growth (ie: percent cover and above-ground biomass harvesting). Above-ground metrics can be accurate proxies for productivity and cover for biomes and vegetation types with RSRs under or near 1, but may not be as useful in biomes and vegetative types with high RSRs. This investigation begins to make connections between what can be measured and seen above-ground using traditional sampling techniques and what may be occurring below-ground in a species with a high RSR. Future studies focused on assessing above and below-ground biomass relationships in the field would be highly informative. The metabolic pathways, cellular regulation mechanisms, and nutrient sequestration potential under various salinity and inundation regimes are vital factors to consider in future investigations, as are the changes in phenology and fertility that were tangentially observed. *In situ* vertical accretion studies may help in understanding long-term response of *S. pungens* to sea-level rise and the potential to adjust marsh elevation.

5 - ASSESSING TEK: APPLYING ECOLOGICAL FRAMEWORKS TO HUMAN AND CULTURAL KEYSTONE SPECIES RELATIONSHIPS

This chapter is in fulfillment of the minor in “Risk and Uncertainty Quantification in Marine Science”, a National Science Foundation Research Traineeship, with the following mission:

“The ocean is the last great frontier on Earth, a major driver of climate and productivity, and a critical resource for humans and wildlife. Managing ocean resources requires scientists and managers to work seamlessly to understand the top-down effects of human actions and the bottom-up effects of climate-change on the ocean system. The National Science Foundation Research Traineeship (NRT) project at Oregon State University (OSU) prepares a new generation of natural resource scientists and managers who combine mathematics, statistics, and computer science with environmental and social sciences to study, protect, and manage ocean systems. Trainees learn to work in transdisciplinary research groups on significant societal problems using large and ever-expanding data resources. With powerful analytical tools, they will be best equipped to track and study the top-down effects of human actions and the bottom-up effects of climate change on the ocean system.”

(<https://marinerisk.ceoas.oregonstate.edu/>, 7/28/2021)

This chapter was written in partnership with Patricia Halleran, a doctoral student in the department of Anthropology. Although the species of focus in the chapter is salmon, the concept and framework proposed is applicable to other species of traditional and cultural importance. *Schoenoplectus pungens*, the species of interest in this dissertation, is a species of ethnobotanical importance in the Pacific Northwest and is used in basketry. Harvest, preparation, and weaving the rush is important traditional ecological knowledge and is obviously tied to the persistence of *S. pungens* in habitats that overlap with the tribes that utilize it. Elders and members of the Snowhomish tribe have traditionally harvested *S. pungens* for basketry from Bowerman Basin, in Gray’s Harbor, WA. They have harvested from this area due to the morphology of the plant being conducive to higher quality basketry, but they have noted declines in population and changes in morphology in recent years.

Estuaries are economically, culturally, and aesthetically important ecosystems and as such present an opportunity for science and citizens to converge. The field sites in question are heavily utilized recreationally and commercially due to the habitat they provide for game and fisheries species. Therefore, the potential for contact and outreach is very high and could provide an opportunity for

discourse and education. The results of the preceding studies may provide an opportunity for the integration of traditional ecological knowledge and western science. Local tribal members are highly attuned to the morphology and extent of current populations of *S. pungens* in Gray's Harbor, WA. The clear negative impact of salinity, especially when paired with tidal inundation, may help local land managers mitigate changes by either allowing increased freshwater inputs during the growing season or establishment of desirable phenotypes in new areas with favorable conditions. The insight gained from this study could result in native plant nurseries geared towards restoration producing location-specific genotypes. The results of this study could show that restoration projects should use specific 'genetic mixes' or local genotypes for higher long term success. Local land managers and state agencies could work together to catalogue and source genotypes for restoration projects with specific goals, such as maintenance of traditional ecological knowledge.

5.1 INTRODUCTION

We live in a time when global environmental degradation threatens the existence of not only biodiversity, but cultural diversity as well. Human society has had a quantifiable impact on the natural world since at least the Age of Exploration, and particularly so since the Industrial Revolution. Every year thousands of plant and animal species go extinct, fragile ecosystems collapse, and disruption in normal weather patterns cause resource wars, loss of coastal territories and traditional lands, and immeasurable human suffering. Loss of important species that have sustained human communities for generations as a result of anthropogenic disturbances will no doubt add to the challenges Indigenous communities and others face as access to land and resources becomes less certain.

Traditional lands are repositories of Indigenous peoples' history, culture, and ancestral knowledge dating back from time immemorial to the present day. Written in the geographical features of a landscape is the very identity of a people who share a deep connection to place and the many species that live there. This link between culture and ecology is often referred to as Traditional Ecological Knowledge (TEK). Although there are many definitions of TEK due to the diversity of cultural groups as well as biophysical diversity, Berkes (2018) broadly defines TEK as the "cumulative body of knowledge, practice, and belief, evolving by adaptive processes and handed down through generations by cultural transmission, about the relationship of living beings (including humans) with one another and with their environment" (8). TEK is a dynamic,

culturally unique, and habitat-specific set of knowledge that includes an intimate and detailed understanding of the animals, plants, and natural phenomena of the land, while also involving a multitude of practices and technologies a cultural group has used to adapt to an area over time and sustainably manage their natural resources (Turner 1988).

The product of our research seeks to include factors of cultural importance to the current Endangered Species Act (ESA) risk assessment framework. Our current legislative conceptualization of extinction risk as outlined in the ESA of 1973 considers risk as a categorical, rarity-based, and national to global-scale phenomenon. This Act put in place a system of protection as the nation began to recognize natural heritage as being of “esthetic, ecological, educational, recreation, and scientific value” (ESA, 1973) and that this natural heritage was in peril of extinction. However, what this definition excludes is the many species that are considered the natural heritage of Indigenous and traditional communities - species that have sustained their cultures, economies, spiritual practices, and entire worldview and way of life for generations.

This new framework will implement the familiar system of rank calculators but will apply metrics and factors drawn from such concepts as traditional ecological knowledge (TEK), cultural keystone species (CKS), and elements of coupled human-species interactions. This holistic approach to endangered species conservation prioritization could result in more informed actions and funding decisions that align societal and cultural concerns with environmental and ecological ones. Not only will this approach give more ‘power’ to protective legislation, it will enfranchise and engage a larger portion of the population - particularly disenfranchised communities.

5.2 BACKGROUND

5.2.1 Traditional Ecological Knowledge (TEK) and Cultural Keystone Species

The study of TEK begins with the empirical knowledge of identifying and classifying species, while also connecting science and culture to assist people in understanding ecological processes as well as their personal and collective relationship and responsibility to the environment (Berkes 2018, Houde 2007, Tang and Gavin 2016). TEK includes both practice, or the way a people exercise their livelihood, as well as the beliefs, values, and environmental

ethics they share. Although Indigenous and traditional peoples have applied, transmitted, preserved, and protected TEK for generations, scientists and agency representatives throughout North America and elsewhere only began to formally recognize TEK in the 1990s (Dudgeon and Berken 2006). However, non-Indigenous scientists and other experts are increasingly looking to TEK, particularly in resource management areas, to help solve the complex environmental problems of our times. As Menzies (2006) points out, “TEK is held as a beacon of hope [and] is being put forward as the solution to a myriad of problems created by industrial resource extraction and intensive factory-style agriculture” (88).

It is important to keep in mind that there are both similarities and differences between Western science and traditional science; however, understanding the common ground between them could help preserve and protect the environment and natural resources, change policies, strengthen communities that are often marginalized, and lead to restorative justice for Indigenous and traditional peoples who are impacted the most when it comes to environmental degradation and climate change. The following diagram compares and contrasts TEK and science, while also pointing out the commonalities they share (Figure 1).

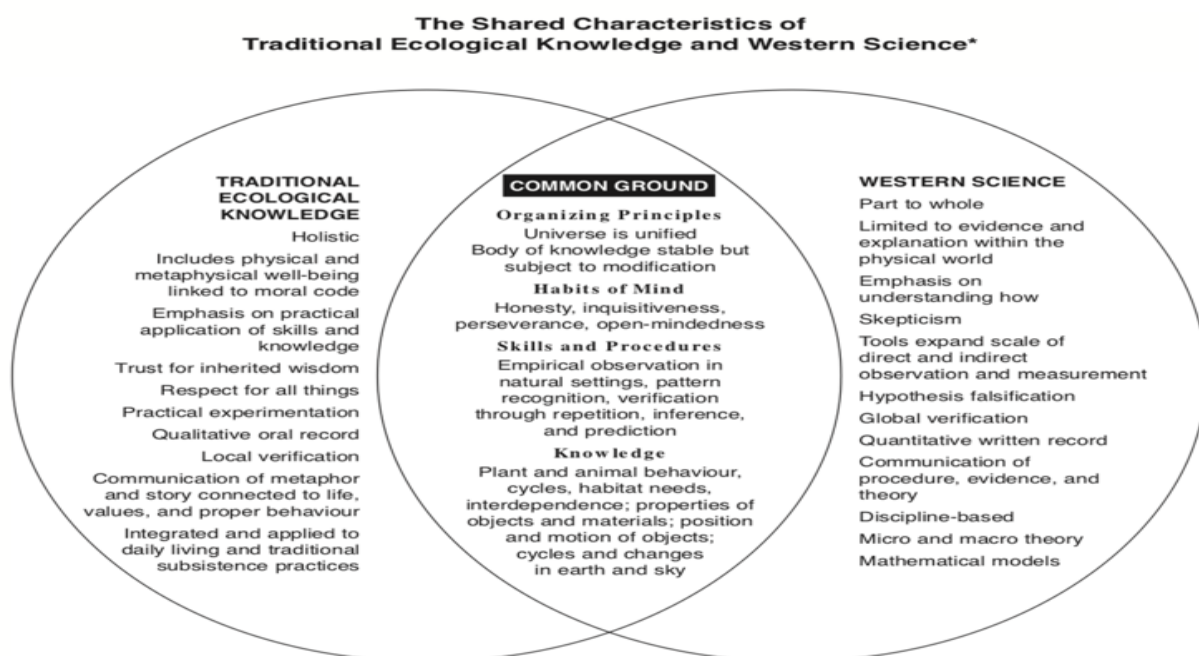


Figure 5.1: Handbook for Culturally responsible Science Curriculum. Stephens, Sidney 2000

By understanding the differences as well as the common ground between TEK and Western science, important natural resources have a better chance of being protected. As the above diagram shows, each epistemology offers invaluable knowledge and expertise that when combined, could help resolve some of the most critical environmental challenges of our times. As previously noted, TEK is multifaceted and complex, and each cultural group defines what TEK means to them. However, there is one common issue that is often overlooked by decision makers - the conservation of species that a cultural group considers a foundation to their way of life.

Cultural keystone species (CKS) play a significant role in the lives and livelihoods of many Indigenous groups, and are defined as animals and plants that are critical to the well-being and security of a community due to the cultural, social, psychological, spiritual, and symbolic benefits they provide (Cristancho and Vinning 2004). While keystone species are “species whose impact on its community or ecosystem is large, and disproportionately large relative to its abundance” (Power et al. 1996, 609), CKS form the contextual foundations of cultural groups, and play fundamental roles in meeting the nutritional, medicinal, religious, and material needs of a people. These species also figure prominently in a culture’s vocabulary, language, and oral histories and narratives, as well as in spiritual and ceremonial practices (Garibaldi and Turner 2004). Just as keystone species are essential to the function of a particular ecosystem and are not temporally or spatially universal, so too are cultural keystone species; what might be a cultural keystone species to one community may not be one for another, even those living in close proximity. Moreover, as culture is not a static phenomenon, people’s dependence on different species evolve as their cultural practices and needs evolve (Turner 1988).

The foremost principle that distinguishes a CKS is the central role it plays in defining the cultural identity of a group. However, there is much debate over how to properly classify and characterize a CKS. Garibaldi, an ethnobotanist, and Turner, an ethnobiologist, first introduced

the concept of CKS in 2004 and created a quantitative index in order to evaluate a species' influence within a culture. They measure six typologies for a species to be considered a cultural keystone including:

- Intensity, type, and multiplicity of use
- Naming and terminology in a language, including the use as seasonal or phenological indicators
- Role in narratives, ceremonies, or symbolism
- Persistence and memory of use in relationship to cultural change
- Level of unique position in culture, e.g. it is difficult to replace with other available native species
- Extent to which it provides opportunities for resource acquisition from beyond the territory (2004, 5-6)

Cristancho and Vining (2004) expand on the CKS framework noting that “the CKS concept has special relevance as a parameter of evaluation within the Cultural Impact Assessment framework since the United Nations Environmental Programme has emphasized the strong connection between ecological and cultural preservation in the context of [I]ndigenous communities” (153). Attributes that Cristancho and Vining deem important for a species to be considered a cultural keystone include: the perceived, empirical, and understood ecological and cultural centrality of a species to a people; use, presence, and abundance of a species in the lives of a cultural group; function of a species in the psychological, social, and cultural structure of a group; existence and interaction with other species; psychoactive importance; and the crucial role a species plays in a group's traditional, religious, and sacred knowledge of place (153-158). Cristancho and Vining argue that for a species to be considered a cultural keystone, it must meet most of the following seven indicator conditions:

- The story of the species' origin is tied to the myths, the ancestors, or the origin of a culture

- The species is central to the transmission of cultural knowledge
- The species is indispensable to the major rituals on which a community's stability depends
- The species is either related to or used in activities intended to supply the basic needs of a community such as getting food, constructing shelters, curing illnesses, etc
- The species has significant spiritual or religious value for the culture in which it is embedded
- The species exists physically within the territory that the cultural group inhabits or to which it has access
- The cultural group refers to the species as one of the most important species (2004, 158-159)

Platten and Henfrey (2009) take the concept of CKS one step further and consider a broader definition. They argue that rather than centering only on a particular species, cultural keystone is in fact a complex which includes both material and non-material components of a larger whole by combining “biological species, knowledge, and technical practices” (493). Therefore, the cultural keystone concept, Platten and Henfrey assert, should examine the fundamental roles a species plays within both ecosystems and social systems, while also attempting to understand the interconnectedness between material and culturally-distinctive subjective factors. By looking at the complex “in terms of its structural and organizational role within such cultural or culture-bearing systems”, Platten and Henfrey propose their own set of cultural keystone attributes. These include a complex made up of such features as:

- Traditional Ecological Knowledge
- Effects on social relationships
- Subsistence strategies
- Economic security
- Social and political organization and formation
- Seasonal and daily labor activity schedules
- Perception of identity and wellbeing
- Gender and age-specific roles
- Ties to interpersonal bonds

- Systems of land tenure
- Cultural adaptation to ecological setting
- Ecosystem services
- Resource conservation strategies

The cultural keystone concept can best be examined in much the same way an ecological keystone is understood. Although Garibaldi and Turner (2004) define CKS as a “metaphorical parallel with ecological keystone species” (2), Henfrey and Platten (2009) argue that cultural keystones are not parallel at all but are in fact directly connected with the ecological keystone term. Just as ecological keystones are crucial to the maintenance of ecosystems due to their contribution of energy flow and structural support of other organisms (Power et al. 1996), cultural keystones are often intrinsic to the cultural survival of Indigenous and traditional peoples. As Henfrey and Platten (2009.) posit:

Social-ecological systems incorporate several different levels of structural complexity. Cultural keystones are system elements with non-redundant functions crucial to the maintenance of social-ecological systems at any analytically identified level of complexity...The keystone is not the species itself but a complex of activities, knowledge and cultural norms based around its production or use (491).

The authors note that dependence on cultural keystone species should be looked at functionally rather than through operational terms, and show how cultural keystones impact a myriad of cultural attributes including population density, economic security, agricultural practices, social dynamics, collective labor, regional networking, and commercial exchange, among others.

If a keystone species is removed from an ecosystem, this would lead to cascading effects that may result in irreparable damage to the entire ecosystem (Franco et al. 2014). If a CKS is removed from a human community, this too could lead to adverse impacts that dramatically change or even endanger an entire cultural group (Lepofsky et al. 2017). CKS form the contextual foundation of a cultural group, and due to their significance in a community in which they may have sustained a people for generations, the impacts they have are deeply rooted within the embodiment of their social, cultural, political, and spiritual identity.

Garibaldi and Turner (2004) point out that “all around the globe, humans identify themselves and each other by their cultural and economic affiliations with particular species of plants and animals” (2). Because a people’s livelihood and cultural identity is profoundly intertwined with the landscape, the loss of a CKS could disrupt the entire cultural keystone complex (Henfrey and Platten 2009). For example, salmon was and still is one of, if not the most, important cultural keystone species to many Indigenous communities throughout the Pacific Northwest. Tribes have depended on salmon since time immemorial to meet their nutritional, religious, and cultural needs. The Columbia River Inter-Tribal Fish Commission (2018) point out several reasons why salmon are so integral to their cultural stability and assert that “without salmon returning to our rivers and streams, we would cease to be Indian people”. Some of the reasons salmon are so important to their communities is because:

- Salmon are a part of their spiritual and cultural identity
- Salmon are used in religious ceremonies such as the First Salmon Ceremony, among others
- The return of the salmon each year assures the continuation of *all* life
- Salmon have been and continues to be an essential economic resource and a preferred livelihood
- Salmon habitat are a part of a “sense of place” and tie tribes to the land as stewards. Are indicator species, and the health of salmon populations are directly connected to the health of the entire food web, including humans
- Have been a primary food source for generations, and are an essential component of nutritional health
- As tribal populations grow and return to pre-colonization levels, salmon and other wild foods are even more important for cultural stability
- Traditional values and TEK are passed onto younger generations during the annual salmon harvest, ensuring intergenerational connections and cultural continuity

As important as salmon is to numerous Indigenous communities throughout the Pacific Northwest, anthropogenic disturbances such as overfishing, hydroelectric dams, commercial agriculture, industrialization, resource extraction, pollution, and climate change have drastically reduced their populations throughout the region, leaving many Indigenous communities in cultural, social, and economic turmoil (Montgomery 2003). Moreover, at least 137 species rely on the marine-rich nutrients that salmon adds to ecosystems, making them an essential contributor to both environmental and cultural stability (Wild Salmon Center 2018).

Both oral tradition and the archaeological record indicate that Indigenous peoples in the Pacific Northwest have been dependent on salmon for at least the last ten thousand years (Montgomery 2003). Due to the aforementioned anthropogenic disturbances, however, populations of this important cultural keystone species are a fraction of what they once were. According to Chad and Lackey (2005), Indigenous harvest of salmon prior to white settlement in the Pacific Northwest was estimated to be approximately 10 million pounds or over 5 million salmon annually. Since then salmon populations have steadily declined, dropping to as low as 3-6% of their original populations (Meengs and Lackey 2005). The ramifications of the disappearance of such a significant cultural keystone species for Indigenous communities is widespread and can lead to malnutrition, economic uncertainty, and the loss of traditional ceremonies and lifeways (Menzies 2006).

Salmon require cold, clean, and oxygenated waters to survive, and are dependent on the unique and ideal environmental conditions of the Pacific Northwest. Moreover, the entire environment is dependent on salmon, as they are an essential species in the complex food web of the region. As Molinero (n.d) points out,

The land relies on the salmon to bring marine nutrients inland, the key element to keep the food cycle going at all the trophic levels. Without salmon, not only the lives of bears, ospreys, bald eagles, martens, wolverines, frogs, salamanders, and even deer and other herbivores would be vastly different if not impossible, but also the livelihood of trees, the productivity of the forest floor, and the insects that are at the base of the food chain would be imperiled without the energetic input of salmon (4).

Simply put, the Pacific Northwest would not be what it is without salmon. Moreover, the symbiotic relationship between Indigenous peoples and salmon that evolved over thousands of years points not only to the sustainable management practices of tribal peoples that protected this essential natural resource for millennia, but should also direct our collective action to repair salmon habitat as both an act of restorative justice for Indigenous communities, and the numerous species that depend on salmon for their survival.

Exploring the meaning, significance, potential, and limitations of the cultural keystone species concept is a worthy project in the face of the global environmental challenges of the

modern era. As every society since the dawn of humanity has relied on specific species to fulfill fundamental roles in their physical and cultural survival, understanding our dependency on them can help us better understand and prepare for our own survival as a species. Understanding the significance of cultural keystone species and how they contribute to the stability of a cultural group is the first step. Garibaldi and Turner (2004) argue that it is important to first consider the three issues of scale when analyzing cultural keystones to fully grasp their significance.

First, cultural keystones vary depending on a temporal scale. As the authors note, “[b]oth seasonal markers, such as phenological cues, and longer-term historical markers, such as ceremonies or other rituals that strengthen cultural cohesiveness, facilitate the cultural continuity of landscape use and management” (6). Throughout the Pacific Northwest, the return of salmon was and still is a culturally significant event in which many tribes hold ceremonies to celebrate. As Montgomery (2003) discusses, “the first fish caught was honored as the First Salmon and either was shared among community members or was ritually eaten by a shaman” (45). The bones of the First Salmon are then returned to the water and are often buried in the riverbed to show respect for the salmon nation. These seasonal and annual ceremonies, as Garibaldi and Turner point out, help “people renew their cultural identity on a short-term seasonal scale and on a longer time scale spanning multiple generations” as traditional knowledge is passed down.

Second, cultural keystones vary on a spatial scale. What is considered to be a cultural keystone to one group may not in fact be one for another group, even those residing in close proximity to one another. Garibaldi and Turner explain this further, pointing out that many factors determine a CKS, and that “availability of resources, plant community structure, and proximity to other cultural keystone species all affect the significance a community places on a particular organism” (7). One important issue the authors address is that cultural keystones are not bound by traditional territory either, and “may be viewed on joint temporal and geographic scales” (6), as is the case with seasonal runs of salmon, or specific plants located in different locations at different times of the year.

Lastly, Garibaldi and Turner explain how cultural keystone species vary on a social scale and may be determined by an individual’s social standing within a cultural group, such as for elders or other tribal leaders. The significance of a cultural keystone could also vary from individual to individual and be dependent on a person’s personal connection to a species. The authors argue that even those species that may be considered “small-scale” keystone species due

to their importance on an individual rather than group scale should “not undermine the importance of such species in maintaining and reflecting wellbeing and identity within that context” (7). Since elders and other tribal leaders play an immense role in the cohesion of a cultural group, protecting CKS that are important to them in turn helps protect the entire community.

These spatial, temporal, and social scales are not seen as separate, rather, they operate on a continuum. An important final point the authors make when discussing these scales is that applying the CKS framework to Western societies may prove to be too challenging, as multiple cultural groups often converge with one another in this context. They argue that the CKS framework works best when applying it to Indigenous communities that have existed in a specific location for generations, and who have a long-term connection with their environment and the species that make up an ecosystem.

The potential that the CKS model has in both conservation research, however, is promising. Even in the midst of the environmental challenges we collectively face as a global community much can be done both to preserve what is left, and to reclaim and restore what has already been damaged. As Garibaldi (2009) points out, “restoration can support ecological parameters such as system function, stability, and integrity, while simultaneously renewing and supporting the cultural beliefs and practices that are integrated with the landscape and species being restored” (328). By recognizing that humans are not separate from nature, but that we are in fact a part of it, the potential to right the wrongs of past mismanagement practices and cultural injustice is key to not only our own survival, but the survival of biodiversity, human diversity, and the planet as a whole.

5.2.2 Cultural Keystone Species and Human Rights

Native Americans endured an incredible amount of suffering and loss as a result of Euro-American colonization, and to this day they continue to fight for their traditional territories and sovereign rights. The liberties they do have have been hard won, and many of the basic human rights afforded to all other American citizens, such as control over their deceased and religious freedoms, have only been implemented fairly recently. The Civil Rights Movement inspired a new generation of people from various backgrounds in their struggle for equal rights and cultural freedoms. Although Indigenous peoples have always played an empowered and active role in

liberating their communities from racial injustice, the Pan-Indian and Red Power movements of the late 1960s became an important catalyst for social change in the United States. These movements helped to expose the pervasive exploitation, mistreatment, and lack of legal protection Native Americans had endured since colonial times, while also fighting for their rights as sovereign nations to forge important changes in human rights policies that affected their quality of life (Fine-Dare 2002:16).

There are 562 federally recognized tribes in the United States with treaty rights protected by the Constitution. These rights often include the right to hunt, fish, and gather in perpetuity in their traditional territories. However, treaties have often been broken and in the 1950s many tribes lost their federal recognition which impacted access to their traditional lands and natural resources as well (King 2013). Through the strength and unity of a cultural revitalization movement in the last few decades of the 20th century, Indigenous peoples in the U.S. put pressure on the government to pass several important laws to protect their communities and receive federal recognition once again. Two of the most important laws pertaining to the basic human rights of Native peoples were the American Indian Religious Freedom Act (AIRFA) and the Native American Graves Protection and Repatriation Act (NAGPRA).

With the passing of AIRFA in 1978, Congress implemented a policy that would “respect and protect the inherent rights of Indian tribes to the free exercise of their traditional religions” (as cited by King 2013:301). Although AIRFA was a critical law in favor of Indigenous self-determination, it did not address the religious customs or ceremonies pertaining to the treatment of their dead, or their access to natural resources, such as sacred plants, needed in their ceremonies. Deloria (2000) believed AIRFA failed to consider the fundamental religious freedoms of Indigenous peoples because it ultimately did not formally address the religious and cultural differences of American Indians. Under AIRFA, however, tribes were finally legally allowed to use some of their most sacred plants, such as peyote. Considering that many cultural keystone species are used in ceremony, including salmon, one could argue that at the very least CKS used for religious and spiritual purposes should receive protection during the risk assessment process.

Until 1990 tribes had no legal rights to their deceased or sacred cultural items. NAGPA changed that to a large extent, although much still gets overlooked in the risk assessment process when it comes to sacred sites and culturally significant artifacts. The National Museum of the American Indian Act (NMAIA) of 1989 created the National Museum of the American Indian within the Smithsonian. NMAIA required the Smithsonian Institution, the nation's largest repository for American Indian human remains and cultural items, to inventory, identify, and when possible, repatriate human remains and funerary objects to the appropriate Indigenous nation. NMAIA was established and designed to enhance Indigenous studies, to preserve and exhibit Native American objects, and to provide a venue for Indigenous research and study programs (National Museum of the American Indian 2014). NMAIA set an extremely important precedent regarding repatriation and paved the way for the most important law to defend the human rights of Indigenous peoples and their dead: the Native American Graves Protection and Repatriation Act.

The Native American Graves Protection and Repatriation Act (NAGPRA; 25 U.S.C.3001 et seq.) is a federal law passed by the United States Congress in 1990. NAGPRA asserts the rights of Native Americans, Alaskan Natives, Native Hawaiian organizations, and their lineal descendants to the respectful treatment and repatriation of human remains, funerary objects, sacred cultural items, and items of cultural patrimony (National Parks Service 2014). All federal agencies, as well as public and private museums and other institutions that receive federal funding, are subject to NAGPRA. Agencies and institutions are required by law to inventory and summarize their collections, consult with tribes and organizations, publish a notice in the Federal Register when determining that remains or cultural items in their possession are NAGPRA eligible, and must attempt to repatriate these cultural items when possible. NAGPRA must also be considered whenever a development project disturbs the landscape where Native American graves and cultural items may be found.

The passing of NAGPRA was a monumental moment for both tribes and the United States. For Native peoples, it was a long overdue recognition of their human and constitutional right to control the remains of their ancestors, their sacred cultural items, and some of their sacred sites. For the United States, NAGPRA represented an important first step in ending the

injustice committed against Indigenous peoples, was a profound symbol of restorative justice, and was one of the most important human rights laws ever enacted by the U.S. Congress.

Although the aforementioned laws that are followed prior to development certainly protect many cultural resources, including archaeological sites, Native American graves, and cultural artifacts, much is overlooked in the cultural impact assessment process. Culture is a complex mosaic that includes systems of knowledge, beliefs, values, norms, patterns of behavior, material objects, structures of power, and interaction with the biophysical world. Culture is also created, learned, and shared by a group of people over time. As such, cultural impact assessments must take into consideration the complexity of a cultural group, while paying special consideration to the unique rights of Indigenous peoples who may be impacted by a development. As King (2013) points out, “cultural resource management...needs to deal with management of the *whole cultural environment* and the effects of contemporary plans and decisions on that environment in all its aspects” (8). What is excluded from most cultural and social impact assessments is people’s dependency on, and inalienable right to, the species that sustain their physical, mental, and spiritual wellbeing and the landscapes that define the essence of their being.

5.3 EXTINCTION

The concept of extinction is fairly recent in Western thought, as it was believed God’s creation was perfection and to have species extinction would tamper with the “completeness of nature” (Rowland, 2009). The shift in scientific thought came about following investigation of the fossil record (Lyells, 1854), although it would require further exploration of the known world before the level of certainty would allow extinction to become accepted. Uncertainty about the world’s unexplored areas meant that new organisms found in early fossil records were assumed to exist ‘somewhere out there’ and that local extinction (extirpation), rather than global extinction, made this possible (Lyells, 1854). New paradigms began to emerge to explain incongruities such as the “former-worlds” view or that of “New World degeneracy” (Buffon, 1788). As scientists, and the public, came to explore the unknown ‘wildernesses’ of the world, it became more unlikely that the unfamiliar megafauna, plants, and other organisms present in the fossil record were still present on earth (Lyells, 1854). This, coupled with new plausible

hypotheses for mechanisms of extinction and its role in the new concept of evolution, eventually swayed an uncertain public to the view that extinction was not only possible, but undeniable (Mayr, 1982). The concept of extinction opened a veritable Pandora's box of uncertainties which would eventually permeate and ripple through virtually all sectors of society, from science to religion, agriculture to industry, and politics and society.

Our understanding of the causes of extinction forms the nexus of many disciplines including: physiology/anatomy, population ecology, genetics, community dynamics, geology, and behavior as well as theology and cultural systems. "Extinction science" or "Conservation Biology" is a prime example of transdisciplinarity and draws from myriad fields within the sciences (Soulé, 1985). Early extinction theories revolved around catastrophic events by famous French naturalist George Cuvier in 1817. Lyell, on the other hand, suggested that a constant background level of extinction was present and was reflected in the fossil record. Lyell's close friend, Charles Darwin, would put forth his hypothesis of descent with modification through natural selection which relied on extinction as a primary mechanism for evolution. Importantly, extinction became inextricably linked to speciation, often providing a mechanism combined with niche specialization for the maintenance of biodiversity (Hutchinson, 1959, Levin and HilleResLambers, 2009).

Current understanding of extinction is that any species that cannot survive to reproduce in its environment and cannot move to a new environment, will become extinct. Therefore, any factor that renders net decrease higher than net increase will result in extinction over time (Mittelbach, 2012). Global extinction is defined within conservation biology as the termination of a species, generally when the last individual of that species dies (Mittelbach, 2012). These determinations often happen retrospectively, which is an important consideration within the context of risk and uncertainty and policy/management. Functional extinction may occur before this point, leading to both different levels of risk for certain species but also uncertainty about when that point is reached. Determinations of extinction further require a clear and certain definition of the 'unit of conservation', which can be a species, a subspecies, a subpopulation, a functional group, etc.

5.3.1 Current Legislation

Concern for nature and the perception of extinction risk is often intertwined with a country's trajectory of development (Petulla, 1977; Opie, 1998). In the United States, concern for loss of species may have developed concurrently, or as a result of, increased perceivable anthropogenic impacts on the environment (Petulla, 1977). The speed with which changes to the environment occurred in the late 1800s and early 1900s was facilitated by new technologies, and meant that rapid and extreme change was perceptible during one human lifetime. While many of these changes were perceived as positive, some changes came to be perceived as negative (Petulla, 1977; Opie, 1998; Kline, 2002.). These negative perceptions may have been due to nature and wilderness playing such an important role in the idealization of our national identity and cultural capital (see: Transcendentalism, national parks, manifest destiny, frontier mentality, etc.). The social and cultural importance placed on the environment in the early 1900s has continued to be an important thread within certain Euro-American segments of the population of the United States, with such figures as Muir, Emerson, and Thoreau and such organizations as the Sierra Club and Nature Conservancy becoming integral parts of the cultural and political landscape (Petulla, 1977; Opie, 1998; Hays, 2000; Kline, 2002).

Early conservation efforts focused on preservation of landscapes and parks of exceptional beauty from damaging extractive industries and development. Concurrently, new technology and expanding markets were leading to noticeable declines, and in some cases extinction, of species such as bison, passenger pigeon, Carolina parakeets, eastern salmon, and white-tailed deer (Kolbert, 2014). These declines led to changes in wildland and natural resource management practices including the creation of game wardens, hunting and fishing licenses, and a period of seeking to 'improve' upon nature by introducing game species and managing populations. Concurrently, land conservation efforts received federal and state attention with the founding of state and national parks and reserves (Opie, 1998). These early efforts have had far-reaching implications for management and policy, eventually forming the foundation for important legislation in the mid and late 1900s.

The 1960s and 1970s saw another explosion of environmental concern in response to rapid negatively perceived change, including: spills, explosions, sweeping development, devastated landscapes, and public health concerns (Hays, 2000; Kline, 2002). These concerns resulted in a wave of environmental legislation: The Clean Air Act of 1970, Water Pollution Control Act Amendments of 1972, National Environmental Policy Act (NEPA) 1970, the Marine Protection, Research, and Sanctuaries Act of 1972, the Endangered Species Act of 1973, the Safe Drinking Water Act in 1974, the Resource Conservation and Recovery Act in 1976, the Water Pollution Control Act Amendments of 1977 (Clean Water Act), and the Comprehensive

Environmental Response, Compensation, and Liability Act,(Superfund Act) in 1980. Although all of these Acts contribute to improving habitat quality, NEPA and the ESA are the most important acts in the context of endangered species; the ESA having been specifically enacted in response to public outrage over species loss.

The Endangered Species Act of 1973 was put in to place to provide protection for imperiled species, with the goal of preventing future extinction. The ESA is rooted in ecology and biological disciplines, and is considered “multidisciplinary” (Daub, 2013). Listing determinations must be based on the best available science and while economic factors are not included in the listing of species, economic impacts must be considered when designating critical habitat of a listed species. A species is considered endangered if it “is in danger of extinction throughout all or a significant portion of its range”, while a threatened species “is likely to become an endangered species within the foreseeable future throughout all or a significant portion of its range” (ESA, 1983). Species that are listed as threatened or endangered receive protections under the ESA which includes designating the critical habitat for the species and enforcing ‘take’ prohibitions (Daub, 2013). Take is defined as "harass, harm, pursue, hunt, shoot, wound, kill, trap, capture, or collect, or to attempt to engage in any such conduct." According to the ESA, a species must be listed under the Endangered Species Act (ESA) if it is threatened or endangered due to any of the following 5 factors:

1. present or threatened destruction, modification, or curtailment of its habitat or range;
2. over-utilization of the species for commercial, recreational, scientific, or educational purposes;
3. disease or predation;
4. inadequacy of existing regulatory mechanisms; and
5. other natural or manmade factors affecting its continued existence.

Although there are currently no policies in place that specifically address the concerns of Indigenous and traditional peoples when it comes to CKS conservation, there are at least two laws under which their protection could fall - the National Environmental Policy Act (NEPA), the National Historic Preservation Act (NHPA), and the American Indian Religious Freedom Act

(AIRFA). NEPA was passed by Congress in 1969 in response to disastrous environmental impacts of federal actions and is considered by many to be an achievement of environmentalism of the 1960s. Unlike the ESA, NEPA has no permitting requirements and does not stop actions, instead it focuses on “informed decision making” and disclosure of decisions and possible environmental impacts to public. NEPA requires an in-depth assessment by federal agencies of the environmental impacts of any recommended federal action that could “significantly [affect] the quality of the human environment”, while also calling for an integrated use of the social sciences in evaluating effects on human communities. NEPA calls for an “integrated evaluation” of possible environmental impacts to enable consideration of environmental impacts and consequences of any action “significantly affecting the human environment.” Agencies are required to provide an Environmental Impact Statement (EIS) that includes a full disclosure of both the positive and negative impacts of a proposal, while also providing alternatives if significant risks to the environment or human communities are expected (Environmental Protection Agency 2018). Along with documenting potential environmental impacts, an EIS must explore the “aesthetic, cultural, economic, social, and health effects, whether direct, indirect, or cumulative” as it pertains to the human environment (NEPA 40 Code of Federal Regulations 1508.8).

One way an EIS attempts to determine the impacts on human communities is by conducting a social impact assessment (SIA). Unlike environmental impacts which begin to occur the moment the ground is disturbed, social impacts start the moment an activity or development is proposed due to the psychological effects it may have on a cultural group. The Interorganizational Committee on Guidelines and Principles for Social Impact Assessment (1994) define social impacts as,

The consequences to human populations of any public or private action that alter the ways in which people live, work, play, relate to one another, organize to meet their needs and generally cope as members of society. The term also includes cultural impacts involving changes to norms, values, and beliefs that guide and rationalize their cognition

of themselves and their society (1).

SIAs are explicitly future-oriented and seek to assess the consequences of policy changes or government actions before they occur. They are carried out with the intent of not only understanding how human communities could be affected, but also to mitigate probable and undesirable social outcomes (King 2013).

Although public input is a required component of any impact assessment, the public's concerns are often not addressed. Perhaps this is nowhere more apparent than the use of eminent domain to expropriate private property, or the illegal seizure of unceded traditional territories for public use or corporate development. As consultants are often hired privately by an industry seeking to develop public, private, or tribal land, "expert opinion" regarding the impacts on human communities runs the risk of being biased and leaving little room for public needs, concerns, and opinions to be taken seriously. As Westman (2013) argues,

One result of SIA and EIA processes is that consultants and their writings on the future assume an authority which takes discussion of the future out of the political arena and places it solidly in the technical arena, rendering debates open to technical interventions, but not to political, legal, or popular challenge. In this technical sphere, knowledge and concerns of indigenous people may be written off as community "perspectives". This is accomplished in part by privileging scientific knowledge and in part by making development seem inevitable (114).

By including the expertise of Indigenous communities during the risk assessment process under NEPA, federal and state agencies could also learn how to protect the cultural keystone species they depend on.

Under the National Historic Preservation Act (NHPA) passed by Congress in 1966, cultural resource impact assessments are also included in the EIS process. NHPA, however, only addresses “historic property” rather than all cultural resources, which is defined as,

Any prehistoric or historic district, site, building, structure or object included in, or eligible for inclusion on the National Register [of Historic Places], including artifacts, records, and material remains related to such property (NHPA section 310(8), cited by King 2013).

Section 106 of NHPA requires federal agencies to consider any impacts a project or development might have on historic properties, and to undergo a review process for all federally funded and/or federally permitted projects. Like NEPA, Section 106 requires scoping while determining the areas of potential effects within a project zone. This includes secondary physical effects, visual effects, auditory effects, sociocultural effects, and impacts on culturally significant natural resources including plant and animal species (King 2013, 123). The protection of cultural keystone species could fall under Section 106 if agencies seriously examined the combined impacts a development may have on Indigenous communities.

5.4 FRAMEWORK DEVELOPMENT

Current legislative conceptualization of extinction risk as outlined in the Endangered Species Act of 1973 (ESA) considers risk in a categorical, rarity-based, and national to global-scale phenomenon. Species are ranked into categories of “endangered”, “threatened”, or “least concern”, which then translates into various protection and conservation prioritizations; influencing funding decisions and federal and state plans for species recovery and habitat conservation and restoration.

J.R. Clark, former director of the US Fish and Wildlife Service under the Clinton administration, mentions in his opinion piece in a 2013 issue of *BioScience*, that there are several opportunities for improvement in the national endangered species programs, among which is prioritization of recovery and resource allocation. This opportunity is especially important given

the often overlooked reality that funding “has never been adequate to recover all listed species” which should logically give rise to a transparent and well-defined process of prioritization (Clark, 2013). Currently, no system of prioritization is in place and funding is based on logistical and institutional factors (Clark, 2013). Clark posits that changes to prioritization and resource allocation could be both the most difficult and the most transformative. While Clark is referring to agencies prioritizing based on biological factors, incorporating cultural factors into prioritization could likewise yield transformative results, and the following framework is an initial effort in this direction.

Exploration of NatureServe and IUCN rank calculators suggests that only ecological, population, and habitat factors are considered, eliminating virtually any coupled human-nature or cultural importance components. Therefore, our framework of risk assessment seeks to apply coupled human-natural systems science approach to improve TEK and CKS assessment and protection.

5.4.1 Existing frameworks and creating a shared lexicon

There has been a considerable effort to categorize and quantify risk of species extinction. Red lists, such as that published by the IUCN, have been used since the 1960s as a way to highlight and publicize globally threatened species. Goals of the red lists were to “raise awareness and help direct conservation actions for species”, with the stated goals of the IUCN red list being “1) to provide a global index of the state of degeneration of biodiversity and 2) identify and document those species most in need of conservation attention if global extinction rates are to be reduced” (IUCN, 1996). The implied assumption, or mental model, of these goals is that biodiversity loss is a negative outcome and that extinction should be reduced.

Criteria for listing species were developed and intended for use in categorizing the majority of described species based on “fundamental biological mechanisms that underlie population dynamics and extinction” (Mace et al. 2008). The diversity of threats and differences in the biology of species required that factors be broadly applicable and flexible while maintaining “consistency, transparency, and validity” (Mace et al. 2008). Factors resulted from the amalgamation of criteria from specialists in diverse taxons (invertebrates, vertebrates, plants)

and focus on elements of natural and human systems that lead to population loss and decrease in range size (Mace et al. 2008). IUCN assessment of threats was largely based on subjective factors and early attempts to classify threats confounded severity of threat, likelihood of extinction, causes of threat, and population vulnerability (Mace et al. 2008). The criteria have undergone iterative refinement in an effort to standardize methods and data sources to increase consistency. Choices for factor inclusion are the result of collaborative research efforts and best available science (Master et al, 2012). Notwithstanding, there are inevitable generalizations inherent in this system of categorization that can influence the applicability of listing. Therefore, red lists should be viewed as attempts to summarize in a quantifiable, replicable, and comparable tool for prioritization and development of conservation targets and actions. When viewed in this way, red lists can be a useful and widely comprehensible framework for communicating risk and motivating policy.

The NatureServe categorical risk assessment framework likewise consists of scientifically agreed upon criteria, specifically eight core “status rank factors” within three categories: rarity, trends, and threats (see Appendix I, Master et al, 2012). Rarity consists of range extent, area of occupancy, population, number of occurrences, number of occurrences or percent area with good viability/ecological integrity, and environmental specificity. Trends consist of long and short term trends, while threats consist of threats and intrinsic vulnerability. Each factor is scaled and weighted according to “impact on risk” which allows partitioning contribution. Importantly, “weights assigned to individual factors reflect their **perceived** influence on extinction/elimination or extirpation risk for the element” but the greatest weight is given to rarity factors (Master et al, 2012).

5.4.2 Methods

A transdisciplinary approach was implemented for the development of the framework for assessing risk for endangerment of TEK (Appendix II). The effort was initially based on frameworks from species conservation status assessments currently in use, including the NatureServe categorical risk assessment framework, the International Union for Conservation of Nature (IUCN) red lists, and ESA species listing protocols. These existing systems were developed as a method of assessing conservation status of species and, eventually, to aid in

prioritizing conservation actions. The framework of factors, threats, and trends developed herein and the adapted conservation lexicon is the synthesis of ecology, policy, and anthropology. The process of categorizing factors and finding a shared lexicon took considerable time and reciprocal learning.

Because environmental anthropologists seek to find solutions to the contemporary ecological challenges of the modern era and advocate on behalf of vulnerable communities, and because environmental scientists know that conservation is dependent on a thorough understanding of how people perceive and value the natural world, this framework and transdisciplinary collaboration bridged two separate yet intertwined disciplines together to at the very least begin a conversation on conservation of endangered species, cultural keystone species, and traditional ecological knowledge.

TEK and CKS literature was reviewed to find analogous factors, threats, and trends where possible, and creating new classifications when existing conceptual frameworks proved constraining. In order to guide the process of identifying threats, Salafsky et al.'s guidelines were taken into consideration (2007, Table 1) as well as existing threat impacts to TEK developed by Tang and Gavin (2016) (Table 2).

Table 1: Characteristics of an ideal classification of threats (Salafsky, 2007)	
Simple	Clear language, examples, understandable by all practitioners
Hierarchical	Creates a logical way of grouping items that are related to one another to facilitate use of the classification and meaningful analyses at different levels
Comprehensive	Contains all possible items, at least at higher levels of the hierarchy

Consistent	Ensures that entries at a given level of the classification are of the same type
Expandible	Enables new items to be added to the classification if they are discovered
Exclusive	Allows any given item to only be place in one cell within the hierarchy
Scalable	Permits the same terms to be used at all geographic scales

Table 5.2: Threat impact generated by considering scope and severity of major threats	
Direct Threats to TEK (Tang and Gavin 2016)	Underlying Threats to TEK
Loss of pathways of transmission	Government policy and legislation
Change of traditional livelihood practices	Contact with other cultural groups
Change of traditional religion and beliefs	Influence of outside market
Change of environment and natural resources	Colonization
Loss of traditional rights	Relocation
Change of traditional institutions	Marginalization of dominant societies
	War, military, occupation and population decline
	Migration
	Economic development pressure

5.5 DISCUSSION

Conservation and extinction reduce down to the premise that extinction results when mortality and emigration are larger than birth and immigration (Gotelli and Ellison, 2004; Mittlebach 2012). The risk factors are defined by the mechanisms identified for each side of this basic inequality. For species, this is represented by differential equations of population growth. Therefore, the extinction risk criteria are based on the declining population paradigm and the small population paradigm (Caughley, 1994). In order to define the risk to TEK and CKS, identifying the mechanisms of “birth” and “death” resulted in a list of factors contributing to

extinction risk. Although equivalent paradigms do not exist within TEK literature, we have identified four primary elements that are fundamental to TEK: knowledge, practice, relationships, and the population of practitioners. This requires a simplification of cultural factors which may be rendered useless through oversimplification but may yield some interesting insights.

For the purposes of this effort, risk in the context of species management is defined as the probability that the conservation goals for a conservation target (species, subpopulation, etc.) are not achieved, which in most cases would involve avoiding extinction and/or maintaining sustainable wild-breeding populations. Under this definition, conservation status assessments and population models are in and of themselves risk assessments as they calculate the probability of population persistence (sometimes adding stochasticity or uncertainty factors) (Soule, 1985). Species populations are generally determined by modelling population dynamics based on available census data, life history, and reproduction (Gotelli, 2004). These models rely on data that is often uncertain notwithstanding it being the ‘best available science’. Additional uncertainty surrounds the fundamental structure of a given ecosystem, including ignorance regarding: interspecific and intraspecific interactions, spatial and temporal heterogeneity, and socio-economic and management dynamics within the human community (Charles, 1998). Some model parameters can be assessed with more certainty than others but the compounded uncertainty is difficult to incorporate into the models themselves. These parameter uncertainties can be reduced over time by continuing to research and incorporate knowledge gained (Charles, 1998). All of these sources of uncertainty combine to make it difficult to assess populations and ultimately renders the decisions made under this uncertainty inherently risky.

Within existing frameworks the unit of conservation or conservation targets can be the species, population, ecosystem or habitat range (Appendix IV, Salfsky et al. 2007). The proposed framework contains factors, threats, and trends (Table 3). Factors combine to give assessors a current status and description of the TEK or CKS in question and include intrinsic and contributing factors. Threats and intrinsic vulnerability are assessed based on expert opinion and includes scope, severity, impact, and timing of the threat in question. The final component encompasses short and long term trends, which requires expert analysis of past data as well as model projections utilizing best available data on the threats identified.

Table 5.3: Conceptual Framework Summary

Factors: Factors give a current status and description	Intrinsic
	Contributing factors
Threats: scope, severity, impact, timing	Threats
	Intrinsic Vulnerability
Trends: require analysis of past data and projections with best available data regarding the threats	Long term
	Short term

Factors, threats, and trends combine to give an assessment of the current status of TEK in question.

Factors that contribute to risk and vulnerability of TEK include both external and internal influences (Appendix V and Appendix VI). Within the proposed TEK framework, TEK is analogous to a species which has four component factors: knowledge, practice, relationships, and population. A holistic risk assessment of all four components is necessary. Contributing, or underlying, factors can negatively or positively impact factors and direct threats (Appendix IV).

Table 5.4: Contributing factors (amplification)	
Negative	Positive
Crime	Cultural centers
Poverty	language revitalization
Unemployment	archives, oral/history collection
Health and wellbeing	
Colonization	
Stochastic events	
Political unrest	
Climate change	

Common external factors that may result in the loss of TEK include conversion, either forced or voluntary, into a market economy as this not only could lead to the loss of traditional land-use practices, it may further the dependency on capital commodities (Kodirekkala 2015). Modernization is another external factor that poses a risk to TEK and includes formal education, loss of Indigenous languages, and moving away from an ecocultural-centered way of life (Kodirekkala 2015). An example of internal factors include increased population size of a cultural group, or an increase in population size in an area that Indigenous peoples practice TEK, which could result in significant negative changes to the environment. Both external and internal factors risk jeopardizing the TEK complex, preventing Indigenous and traditional peoples from participating and practicing ancestral knowledge that help to stabilize cultural cohesion. In short, both external and internal factors need to be addressed to prevent the erosion of TEK.

5.6 CONCLUSION

Western science has always determined conservation practices within the United States, while assuming that its logic is the ‘best’ epistemological approach to environmental management. As Berkes (2018) argues, however, “this style of conservation has neglected the values, needs, and aspirations of local people, their knowledge and management systems, their institutions, and their worldviews” (261). Therefore, only by accepting and respecting Indigenous Traditional Ecological Knowledge can a new framework for conservation be implemented, one that takes into consideration the importance of cultural keystone species that these communities depend on, while also attempting to preserve the cultural landscape of a people.

A transdisciplinary approach allowed the exploration of highly complex and quantitative social factors within the context of risk assessment frameworks. While only in its nascent stage, the list of threats and factors produced is the result of an intensive and iterative process which sought to distill and simplify highly complex and sensitive social dynamics. While it was not ultimately possible to assign numerical values or weights to factors or threats in this first attempt, future work should seek to further adapt this framework to the NatureServe species calculator. Iterative evaluation of threats to the identified factors of knowledge, practice, relationships, and practitioners with input from a wider audience may result in universally applicable risk factors and threats. This holistic approach to endangered species conservation prioritization by incorporating TEK and CKS risk assessment could result in more informed actions and funding decisions that align societal and cultural concerns with environmental and ecological concerns. Not only will this approach give more ‘power’ to protective legislation, it will enfranchise and engage a larger portion of the population.

Specific plants and animals are important cultural resources to all human groups throughout the world. Their protection and conservation should be considered common practice in any risk assessment process.

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Appendix I: NatureServe Conservation Status Rank Factors

Table 1.
Summary of NatureServe
Conservation Status Rank
Factors.

Factor Category	Subcategory	Factor	Definition
Rarity	Range/ Distribution	Range Extent	Minimum area that can be delimited to encompass all present occurrences of a species or ecosystem, typically excluding extreme disjuncts and vagrancies.
		Area of Occupancy	Area within the range extent that a species or ecosystem actually occupies. For species, area can be estimated by counting the number of occupied cells in a uniform grid. In most cases a grid of size 2x2 km (a cell area of 4 km ²) should be used, but a smaller 1 km ² grid is appropriate for linear ¹ and some other occurrence types. For ecosystems, areas can be measured or estimated directly based on the best available information. Area of Occupancy for ecosystems is assessed based on selecting the typical spatial pattern of the type (small patch, large patch, matrix).
	Abundance/ Condition	Population Size (species only)	The estimated total wild population of a species, occurring in its natural range and based on counts or estimates of the number of individuals that are currently of a reproductive age or stage, or mature and currently non-reproducing. This category is not included in the assessment calculation for annual plants or invertebrates with population sizes that fluctuate greatly from year to year.
		Number of Occurrences	Number of extant locations (stands) of an ecosystem, or discrete areas occupied by a species (typically subpopulations, populations, or metapopulations). ²
		Number of Occurrences or Percent Area with Good Viability/ Ecological Integrity	1) Number of occurrences (locations, stands of an ecosystem, or number of locations, subpopulations, populations, metapopulations of a species) that have excellent-to-good viability or ecological integrity (A or B occurrence ranks), such that there is the likelihood of persistence if current conditions prevail; OR 2) Percent of the total area occupied by a species or ecosystem that has excellent-to-good viability or ecological integrity.
		Environmental Specificity	The degree to which a species or ecosystem depends on a relatively scarce set of habitats, substrates, food types, or other abiotic and/or biotic factors within the overall range. Relatively narrow requirements are thought to increase the vulnerability of a species or ecosystem.

Continued

Factor Category	Subcategory	Factor	Definition
Threats		Overall Threat Impact	Degree to which the integrity of an ecosystem or viability of a species is affected by extrinsic factors (stressors) that degrade integrity or viability, and which are characterized in terms of scope and severity. Threats are typically anthropogenic, having either direct (e.g., habitat destruction) or indirect (e.g., introduction of invasive species) impact.
		Intrinsic Vulnerability	Degree to which intrinsic or inherent characteristics, such as life history or behavior patterns for species, or likelihood of regeneration or recolonization for ecosystems, make it susceptible or resilient to natural or anthropogenic stresses or catastrophes.
Trends		Long-term Trend	Degree of past directional change in population size (for species only), extent of occurrence, area of occupancy, number of occurrences, and/or viability or ecological integrity of occurrences over the long term (ca. 200 years).
		Short-term Trend	Degree of past directional change in population size (for species), extent of occurrence, area of occupancy, number of occurrences, and/or viability or ecological integrity of occurrences in the short-term, considered to be typically within 50 years for ecosystems, or within 10 years or 3 generations, whichever is longer (up to 100 years), for species.

*Table 1. (continued)
Summary of NatureServe
Conservation Status Rank
Factors.*

Appendix II:

Transcribed discussion linking population ecology and TEK (creation of boundary object)

“...Population ecology is study of the distribution and abundance of species. Population ecology models are often based on estimates and methods to account for non-detection, which may not be as much of an issue within the context of TEK. Identifying the conservation unit and quantities of interest may be the more important factors. **Note: population is tied inextricably to resources and geospatial extent. This may not be the case in TEK quantification. ‘Space’ as a resource will need to be explored.**

The ratio between the range extent and area of occupancy is a measure of fragmentation and isolation, a relict of enforced occupancy restriction. Enforced occupancy restrictions could be the species niche requirements or tribal resource areas or reservations.

- Range extent: The area that encompasses all occurrences, but unlike Area of occupancy, there can be areas with the range which have no occurrences
- Area of Occupancy: Points within the extent that are actually occupied.
- Geographic distribution : Where do members of the tribe live? Are they close to resources
- Metapopulation dynamics? A system of subpopulations, any of which can go extinct and later become recolonized
- Connectivity/isolation
 - Intertribal connectivity (hybridizing?): Sharing knowledge and creating new knowledge
 - Intra-tribal: Rescue effect: number of subpopulations (bands)
 - Transmission = transportation corridors? Wildlife corridors in high fragmentation areas?
 - Edge effects: Cultural exchange, assimilation

Abundance and Condition

Population size: how to define population? In conservation biology, and by extension the NatureServe rank calculator, it is the estimate of the total wild population of a given species in its natural range. In population and genetic ecology this definition is generally based on counts or estimates of mature individuals (reproducing and non-reproducing) living in the same place, at the same time, and capable of interbreeding. A further refinement of this concept is the effective population size, N_e , which is the number of reproducing individuals that are contributing to the next generation at a given time and this number is generally lower than the total population number, N . Effective population size is often used as an idealized quantity that is useful for modeling a given quantity of interest (genetic loci, etc.). Effective population, or N_e , calculation makes several assumptions included random mating, simultaneous birth of each new generation, constant population size, and equal numbers of children per parent. Within the context of TEK, utilizing a concept of effective population may yield a better estimate of individuals that can contribute a characteristic of interest to the next generation, in this case, knowledge of a particular practice analogous to a genetic loci, allele, or trait. Additionally, the mechanism of reproduction or transmission to the next generation will not necessarily occur at future timesteps

or be automatically assumed as in population modeling. Transmission of traits or TEK can occur within the same generation or skip generations.

How to identify the effective population size in the context of TEK? A simple census of individuals will not yield an accurate characterization of population number therefore, what are the traits/loci of interest and how can they be estimated within a population? Age structure:

Elders, Young people with interest, Size of tribe: blood quantum/census

Would a patch occupancy model work for modeling TEK?

of occurrences and spatial distribution of viable patches : “Number of extant locations of an ecosystem, or discrete areas occupied by a species, typically subpopulations, populations, or metapopulations.” Number of Occurrences or Percent of area occupied with good viability/ecological integrity (Patch occupancy).

Habitat relationships and resource selection: based on the concept of ecological niche (Hutchinson 1957 classic), in which each species has a set of requirements that must be met for a species to persist, therefore each species has a theoretical N-dimensional hyperniche in which it can exist. Defining all the requirements is virtually impossible but identifying some factors needed for persistence can aid in defining management solutions as well as identifying potential unoccupied patches conducive to persistence/colonization. This approach can also help explore the level of resource use in a given area.

Patch Viability : access, habitat integrity, etc.

Environmental Specificity (used when number of occurrences and area of occupancy is unknown)

- Specialization
 - How many other groups use this species? (Economic dependence, cultural use)
 - How universal is the species appeal?
 - How many other uses/benefits does the species provide?

Some of the main differences between TEK relationships and species that I can notice right off the bat:

- 1)the intelligence/free-will of humans
- 2) Motivation as a threat in humans. In ecological models it is assumed that the ‘motivation’ of species’ behavior is solely driven by survival to reproduction. The persistence of TEK/relationships does not impact survival to reproduction in that same way...how to deal with this?
- 3)Human ability to change the environment to create additional viable patches
- 4)Rarity is the principal factor for species risk but threats and trends may be more important for TEK. Different weighting or scaling?
- ** 5) What counts as survival? **”

Individual Research Matrix

Research question: Does our current legislative conceptualization of extinction risk as a categorical, rarity-based, and global-scale phenomenon reflect our ecological understanding of clonal coastal wetland populations?

Target Audience	Key Values & Concerns	Key Messages	Desired Actions
Government decision makers - Federal	Constantly torn between use and conservation pressures. Concerned with the public perception of their agency. Concerned with maintaining favor with funding and budgeting arms of government as well as influential lobbying and/or donor groups. Depending on their position, they may be accountable or have responsibility for the success of the policy in question. Do not like uncertainty, unexpected results or unprecedented events (especially when being pushed to change the current paradigm). Value consensus, always want a lot of buy-in and data before making decisions; the more unpopular, the more data and certainty required. Value clear success criteria, not the improvement or diminished severity of possible outcomes (especially if uncertain). Probably have some personal interests and connection to nature.		
Government managers - policy implementers	Always concerned with “acceptable, affordable, and achievable.” Need to meet goals, trajectories, expectations, and milestones set by policy makers. Concerned with various ‘success criteria’ that are set explicitly and implicitly by the public (beauty of the area, fish/game abundance, recreation opportunities). Feel the need to validate the utility of their position and the importance/benefit to society. Need to maintain their funding source and budget and so are interested in finding ways to improve their performance based on the criteria of existing policy. Often have to deal with uncertainty regarding policy/political climate, funding, and requirements. Probably have a personal connection to nature.		
Ecologists in academia	Want to answer questions that they find interesting and important, generally narrowly focused. Concerned with research funding, successful publications, and advancing within		

	the university system. Want to accumulate ‘research power’ by expanding their network and access to research resources (equipment, space, man-power, etc.). Value diffusion of their knowledge with others that may have information relevant to their topics/interests. Highly value their perceived expertise. Often comfortable with the nuances of uncertainty, but value continued research/accumulation of data. Usually have their own implicit personal certainties and biases. Often conflicted or do not value allocating time to communicating the relevance, importance, and possible impact of their work to ‘non-experts’. Can be disengaged with, or not value, the complexity of public relations or policy. Can often be conflicted between theoretical and applied approaches. Generally have a deep connection to their research interest.		
Extractive industry directors	Concerned with maintaining profitability, ensuring continued growth/demand, and safeguarding their ability to operate. Do not value any added regulation, complexity, or change in operating procedures that can result in temporary (or permanent) profit losses. All practices are dictated by profit margin, health/safety regulations, and maintaining the favor of the public and government regulators. Value opportunities to improve their image as it can impact regulation of their industry, being perceived as cooperative, culturally/societally important, and ‘good’ by at least a segment of the population. They value maintaining good morale and supply of reliable cheap workforce. Probably don’t have a ‘conservationist’ connection to nature, but may like recreational uses of natural habitats (hunting, fishing, etc.).		
Private sector - Conservation NGOs	NGOs need funding. They value a clear and ‘attractive’ message that is easily understood and appeals deeply to funders. NGOs value connections with interest groups and/or powerful demographic groups. They are concerned with maintaining legitimacy, expertise and capacity to effect change.	Global extinction is a fairly straightforward concept and elicits an	

	NGOs value trust and position and they do not like uncertainty or changes in ‘message’. They value positive exposure, leverage, and power for the organization. NGOs are concerned with representing or providing a tangible/approachable solution while demonstrating their capacity to implement it. They value certain lifestyles, hobbies, activities, and morals that align with their message or help diffuse their ‘brand’. They value beauty in nature, iconic species, and culturally/emotionally impactful visuals (photos, videos, etc.). Probably feel a deep connection to their cause.	emotional response.	
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Team Research Matrix

Research question: Can the “Cultural Keystone Species” concept be an effective transdisciplinary tool for holistic species extinction risk assessment? *Main point: CKS can give local groups increased ‘power’ and voice.*

Target Audience	Key Values & Concerns	Key Messages	Desired Actions
Government decisionmakers – local	Concerned with ‘power’/voice, funding, and career (may have interests in advancement to other branches of government). Value self-determination and self-governance for their community. Concerned with re-election and community support, therefore they need to show results and successes on issues that the community finds important. Value their communities’/constituents’ unique needs and values. Personal concern for improving their immediate living environment and community.		
Government decisionmakers – federal	Getting policy passed, maintaining positive perception, and ensuring on-going cooperation.		
Community decisionmakers – local special interest groups	Feel special connection to place or species. Value the unique characteristics of their community (ecological or human). Concern can be driven by the immediacy of the impact or everyday reminders of change. Value being heard and impacting change/action.		

	Feel urgency and concern while not being overwhelmed with negativity, futility, or complexity.		
Tribal groups - local	Value sovereign rights, self-governance, and maintaining/perpetuating their culture. Concerned with improving their access to and influence over policymaking that impacts their land and/or existence. Value deep cultural and spiritual connection to land/natural environment. Concerned by historic precedents and generally do not value US bureaucracy. Value policies and legislation that grant sovereignty or consideration to tribal rights. Value acknowledgement and resources that are devoted to address their concerns. Do not value policy or legislation that circumvents tribal sovereignty or dictates tribal action.		

- Target Audience
 - what and who is contributing to problem
 - what entities have the most political or social influence
 - what entities may be positively and negatively effected
 - what entities could be involved in implementation
 - what entities pose challenges when information is distributed (who can help or hurt you)
- Key message: the three c's
 - Connecting to existing values (what they already know and believe)
 - Create interest: why should they care, what new information is out there
 - Counter concerns: specific
- Desired Action: What you want to have happen as a result of communication plan, what actions might every TA take?

6 – SUMMARY AND CONCLUSION

Chapter two demonstrated that not only can germination success for certain populations be higher than previous studies suggest, but that ideal conditions for germination may be dictated by source population. The percent germination ranged from a low of roughly 0% (Skagit River, WA) following warm dry stratification to a high of roughly 76% (Big Lagoon, CA following warm dry stratification and Skagit River, WA following cold wet stratification). Ultimately, higher germination rates could be achieved by tailoring stratification to population preferences but implementation of wet and cold stratification is the most consistent method if site of origin is unknown. Future studies should focus on dormancy types and mechanisms in *S. pungens* to improve germination for restoration and field sowing, with germination including bleach scarification combined with temperature and light fluctuation.

Chapter three results show that salinity, inundation, and source population all impact the proportion of fertile culms, maximum height, and the number of culms produced, although to varying extent. The results of the current study showed that high salinity combined with tidal conditions not only reduces the overall number of culms, but also reduced the proportion of culms producing inflorescences. The absence of fertile culms in the first growing season may indicate trade-offs between sexual and asexual reproduction with life-stage, a strategy which was not previously recorded to occur in this species. Fewer culms were produced in the salt treatment but they tended to grow to a similar maximum height as those in fresh and brackish treatments, suggesting that salinity did not result in stunting vertical growth, but in lateral expansion. The number of live culms per plant showed a similar negative interactive effect of high salinity under tidal conditions that was seen in the biomass response (chapter four). The results showed some evidence of local adaptation in culm number and max height as evidenced by between site differences, specifically Coos Bay, OR, Big Lagoon, CA, and Skagit River, CA. Coos Bay, OR seemed to have a lower tolerance to the high salt treatment as it generally had the lowest maximum height and live culm number whereas Skagit River, WA had taller culms but generally fewer of them, especially in brackish and salt treatments. This may indicate a local adaption in the trade-off between lateral and vertical growth for both sites.

Chapter four results showed that in non-tidal conditions *S. pungens* can withstand near seawater salinity concentrations but in tidal conditions at the same salinity *S. pungens* biomass production suffers. The results of the biomass allocation analysis suggest that below-ground biomass is disproportionately important to *S. pungens*, as evidenced by all RSRs being above 1, dramatically so in some cases. The RSR trends in response to salinity were inverse depending on the inundation condition, again highlighting the importance of the interaction between salinity and inundation on *S. pungens*.

Chapter five provides a link between western science, traditional ecological knowledge, and conceptualization of risk of species loss. The proposed framework implements the familiar system of rank calculators but applies metrics and factors drawn from such concepts as traditional ecological knowledge (TEK), cultural keystone species (CKS), and elements of coupled human-species interactions. This holistic approach to endangered species conservation prioritization could result in more informed actions and funding decisions that align societal and cultural concerns with environmental and ecological ones. Not only will this approach give more ‘power’ to protective legislation, it will enfranchise and engage a larger portion of the population - particularly disenfranchised communities.

The cumulative results of chapters three through five show that both local adaption and phenotypic plasticity may play a role in vegetative and reproductive response to salinity and inundation, and that the degree of local adaptation may differ between sites. The results show a fairly strong interaction between inundation and salinity, with tidal conditions paired with high salinity generally being the least favorable conditions for *S. pungens*. These conclusions have important management implications as stands of *S. pungens* may benefit from increased freshwater or carefully timed plantings in areas foreseen to have salinity changes. The clear negative effect of salinity on fertile culm production show that understanding reproduction dynamics, even in species with perceived low sexual reproduction, is important from a conservation perspective. Future studies should focus on meta-population dynamics and sources of genetic variation in clonal wetland species.

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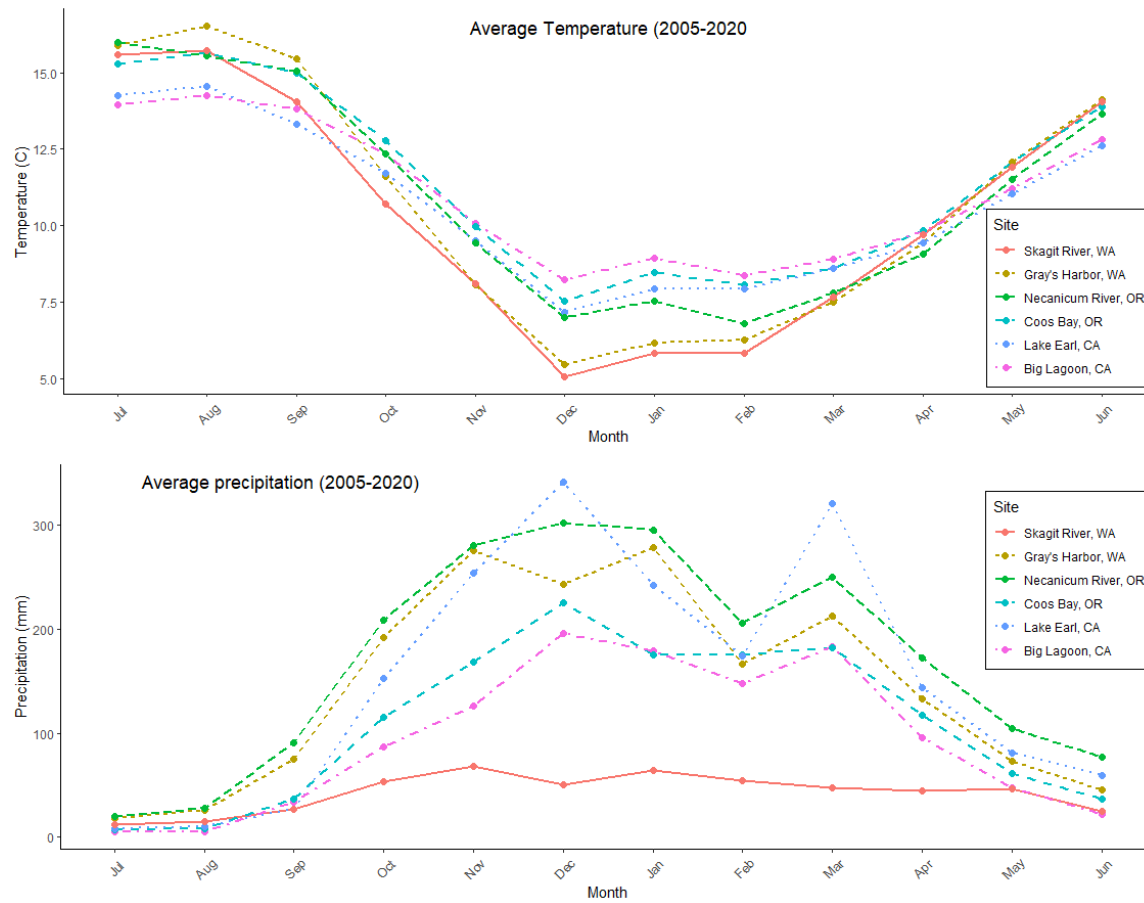
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Appendix I: Site precipitation and temperature at study sites



Appendix II: Mesocosm experimental design

