Eleocharis geniculata (Bent Spike-rush) Great Lakes Plains population: conservation in the face of invasion

by

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Author's declaration

I hereby declare that I am the soul author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

I understand that my thesis may be made electronically available to the public.

Abstract

Eleocharis geniculata is listed as endangered under the Canadian Species at Risk Act, S.C. 2002 and the Ontario Endangered Species Act, S.O. 2007. Despite these listings, the current Ontario government recovery strategy lists the location and population dynamics, habitat requirements and the characterization of threats as knowledge gaps preventing the recovery of the species (Bowles 2010). Comprehensive surveys of *E. geniculata* in the three known critical habitat locations for the Great Lakes Plains population (Cedar Springs, Rondeau Provincial Park, and Long Point National Wildlife Area) have not occurred since 2007. Prior to my thesis work, it was unknown if *E. geniculata* still existed in Ontario. While some information was known about *E. geniculata* habitat requirements, such as its low tolerance for competing vegetation (Bowles 2010), our understanding of *E. geniculata*'s habitat needs was largely qualitative. Further, invasive *Phragmites australis* is currently recognized as the main threat to *E. geniculata*, but this also had not been quantified (Bowles 2010). My objectives were to: 1) search critical habitat to locate and census any extant *E. geniculata* patches; 2) characterize the habitat requirements of *E. geniculata* by quantifying biotic and abiotic site characteristics at extant and recently occupied locations and; 3) characterize the threat and map the presence of invasive *P. australis* adjacent to any *E. geniculata* patches to evaluate the risk that this invasion poses to remaining *E. geniculata*. I conclude that *E. geniculata* still exists in Ontario, but most likely only in the Long Point National Wildlife Area, where it is threatened by invasive *P. australis,* which has spread unchecked for decades. In Long Point National Wildlife Area, I located one patch of *E. geniculata* in 2017 and two patches in 2018, both of which had invasive *P. australis* growing in or adjacent to them. My research confirmed the anecdotal information about habitat requirements, including that *E. geniculata* cannot tolerate competition for light or persistent inundation with standing water > 1 cm in depth. *Eleocharis geniculata* occurred only on bare sandy substrate with low organic content and nutrient levels and relatively high calcium. Critically, my results confirm that invasive *P. australis* is a major threat to *E. geniculata* with its ability to outcompete *E. geniculata*, and its tendency to grow in monoculture stands in the preferred habitat of *E. geniculata*. *Phragmites australis* intercepts between 58 and 98% of incoming photosynthetically active radiation, leaving too little for *E. geniculata* to assimilate carbon via photosynthesis.

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Important note

This document has had location information anonymized to 1 km^2 grids as the location of endangered species is confidential and must be handled in accordance with NHIC sensitive data protocols. Precise location information can be obtained from NHIC by authorized personnel with a legitimate need for the data who have completed data sensitivity training.

1   <u>Literature review and thesis scope</u>

1.1   <u>Wetlands and biodiversity</u>

Wetlands cover less than 1% of the earth's surface, yet they support 6% of described species globally (Dudgeon et al. 2006). Wetlands contain a disproportionality high amount of biodiversity to their area (Dudgeon et al. 2006) as both terrestrial and aquatic species can utilize wetland systems, increasing their biodiversity significantly (Deane et al. 2016). Unfortunately, wetlands are drained, degraded and converted for human use more than any other natural system (Ramsar Convention on Wetlands 2018). The ecosystem services that wetlands provide were often unnoticed, leading to wetland decline (Ramsar Convention on Wetlands 2018). Globally, the loss of wetlands is estimated at between 64 and 71% since 1900 AD (Davidson 2014). However, the importance of wetlands is now realized, with many conventions in place to help protect and conserve these valuable habitats. It is essential to protect biodiversity because a greater variety of species creates a healthy, functioning ecosystem (Gamfeldt et al. 2008). This is because species have different functional roles, or features that affect ecosystem processes (Lavorel and Garnier 2002). Having greater biodiversity increases the likelihood that multiple species perform the same function (Walker 1995). Species performing the same function increases the stability of the ecosystem as each species would have different responses to environmental changes (Walker 1995) such as disturbance. For example, some species become more abundant during heavy grazing than others, allowing those species to continue to perform the ecosystem functions that the previously dominant species did before the disturbance (Walker et al. 1999).

Hydrological changes in wetlands create natural disturbances (Bornette and Amoros 1996, Moran et al. 2008) that increase the number of rare plant species (Deane et al. 2016) and species richness (Pollock et al. 1998, Raulings et al. 2010). Further, Deane et al. (2016) noted that rare species were often negatively associated with common species, and rare species are often associated with rare habitats (Hodgson 1986, Totté et al. 2015). This makes all wetlands conservation priorities as they add to the patchiness and biodiversity of the landscape (Denny 1994). The dynamic nature of wetlands creates variable habitats that allow for such a high diversity of species (Halls 1997).

Environmental factors such as water level and soil texture influence the plant species that can exist in a given habitat (Weiher and Keddy 1995). However, recent studies have shown that environmental filters are not the only mechanism influencing species' ranges (Cadotte and Tucker 2017). Competition is also capable of excluding or facilitating the growth of particular species in an area (Bengtsson et al. 1994). Together, competition and environmental characteristics shape communities (Cadotte and Tucker 2017, Germain et al. 2018). Understanding habitat characteristics such as water levels and soil characteristics and identifying species that co-occur with rare species can assist with locating potentially suitable habitat, even if the rare species is cryptic or has a low detection probability.

1.2   Nutrient availability

Species distributions within wetlands vary depending on the nutrients required by and available to them (Bedford et al. 1999). Nutrient availability can lead to shifts in plant distribution (Kooijman 1992), including species richness and composition (Bedford et al. 1999, Wassen et al. 2003). Nitrogen is often a limiting factor for primary production in wetlands (Bedford et al. 1999). When nitrogen is added through human activities such as farming, primary production increases; however, species richness usually declines (Cornwell and Grubb 2003). The decline in species richness could be caused by increases in soil fertility which allows some individuals to grow larger, preventing other species from establishing (Stevens and Carson 1999). More recently, Lü et al. (2020), found that increases in nitrogen lead to acidification of soil, which reduced species richness in grasslands. Declines in species-rich wetlands is concerning as they are more likely to contain rare species (Johnson and Leopold 1994) and are, therefore, a conservation priority.

1.3   Soil

Soil quality, including conductivity (Li et al. 2017), salinity, and temperature, are vitally important to plant distributions and habitat suitability (Cronk and Fennessy 2001). Levels of soil conductivity vary among different wetland types and influence the dominant species within those wetlands (Rejmankova et al. 1995). Many wetland species are constrained by soil salinity (Janousek and Folger 2014), with some evidence showing that aquatic plants colonize after restoring wetlands with freshwater to reduce salinity (Cui et al. 2009). Soil temperature affects

seedbank germination, resulting in altered vegetation structure, including changes in biomass and stem density of perennial and annual species (Seabloom et al. 1998).

1.4   Light availability

The amount of light reaching vegetation in a wetland can act as an ecological 'filter' to exclude certain species. For example, species with low growing morphologies are positively associated with light levels (Schrautzer and Jensen 2004, Kotowski and Van Diggelen 2004). Additionally, lower light levels can decrease plant biomass (Maurer and Zedler 2002, Perry and Galatowitsch 2004). Light availability at the soil surface especially affects annuals as they require germination from the seedbank. This is because some species need sunlight to reach the seedbank to trigger the germination of seeds near the soil surface (Carta et al. 2013).

1.5   Fluctuating water levels

Fluctuating water levels are vital in sustaining high levels of plant diversity in wetlands (Gathman et al. 2005), although wetlands with moderate water levels encourage higher species richness (Roznere and Titus 2017). This is because minor changes in water levels can affect the success of wetland plants (Roznere and Titus 2017). For example, Roznere and Titus (2017) found that *Sparganium americanum* (Nutt.) accumulated 96% more biomass when growing 3 cm below water than 11 cm above the water. Further, some species have adapted to tolerate only a narrow range of water levels and are unable to germinate outside of that range (Keddy and Ellis 1984). When water levels are high, it can create an abiotic barrier to germination (Fraaije et al. 2015). Alternatively, when water levels are low, water becomes a limiting resource (Fraaije et al. 2015), also excluding certain plant species with low water use efficiency. This is especially true during the germination phase, which influences the resulting composition of species (Fraaije et al. 2015).

1.6   Co-occurring species and phenology

In addition to environmental filters, interspecific competition for resources among species can also help with predicting species communities (Segre et al. 2014) and defining niche boundaries (Letten et al. 2017). Species' ability to coexist and interact with their environment can limit what species can persist within a community (Ferreira et al. 2015, Wang et al. 2015). Understanding

what species commonly coexist and why is also helpful because more common species that co-occur with rare ones can be used to locate potentially suitable habitat. For example, Baumberger et al. (2012) predicted suitable habitat for *Linmonium girardianum* by incorporating common co-occurring species into a species distribution model.

1.7   Climate change

Anthropogenic climate change is a significant threat to global biodiversity (Thomas et al. 2004). Withey and Kooten (2011) estimate that the effects of climate change could reduce the number of wetlands between 7 and 47%. Climate change is affecting temperatures (Alley et al. 2007), hydrological cycles (Ferrati et al. 2005, Havril et al. 2018), and causing more extreme and unpredictable weather patterns to emerge (Chen et al. 2011, Bellard et al. 2012), all resulting in a loss of biodiversity (Thuiller et al. 2005). Davidson (2014) suggests that >70% of wetlands have already been lost or compromised by anthropogenic changes, including climate change. In North America there has been an increase in precipitation since the 1900s (Trenberth et al. 2007). However, Qu and Zhuang (2019) suggest that North America will get drier. In the Great Lakes region, wetland plant communities are influenced by fluctuations in water levels (Wilcox and Nichols 2008) and changes to the hydrological cycle could see an increase in the spread of invasive species. For example, invasive *Typha* x *glauca* dominates when water levels are low (Lishawa et al. 2010), so increased periods of low water depth could encourage its spread. Wetland plant biodiversity will be lost through changes to the hydrological cycle and the loss of suitable habitat.

Changes in species' ranges towards the poles have already been evident (Parmesan 2006). Species at risk will also become more threatened by environmental changes, as suitable habitat becomes unavailable in the parks (Monzón et al. 2011). This can occur at a rapid rate, for example, Thomas et al. (2004) estimates that by 2050, if climate change trends continue, 15 - 37% of species in their study area will be committed to extinction.

1.8   Peripheral populations

Peripheral populations, or populations at the edge of a species' range, were once believed to add little to the resilience of species (Channell 2004). Conservationists often focused on populations

at the center of the species range for protection (Channell 2004). This was supported by the fact that populations at the center of the range are often larger and more connected to each other, increasing genetic diversity (Channell 2004). Populations at the edge, however, often have limited habitat, are more secluded and are more likely to disappear from changing environments (Channell and Lomollno 2000). Channell and Lomollno (2000), tested this theory by studying historical ranges and current ranges of 245 species, finding that 98% of those species still exist in a portion of their peripheral range. Further, it was found that 68% of the species had a greater population in the peripheral range than the center of the range (Channell and Lomollno 2000). This shows that despite having smaller populations and perhaps lower genetic diversity, peripheral populations have the potential to persist for longer than core populations.

Increasingly the uncertainty around the ultimate affect of climate change makes the conservation of populations across the entire range of a species vital, rather than focusing solely on populations at the centre of a species' geographic distribution (Gapare et al. 2005, Leppig and White 2006, Bateman et al. 2015). Though populations at the edge of a species' geographic distribution are typically at a higher risk of extinction (Safriel et al. 1994), such peripheral populations may have novel evolutionary potential through adaptation to abiotic and biotic pressures not faced by the species at the center of its range (Leppig and White 2006). These adaptions could result in genetically distinct populations of both local ecological and evolutionary significance, making threatened peripheral populations of high conservation value (Leppig and White 2006). For example, Brzosko et al. (2009), studied *Cypripedium calceolus* (L.), which has a center range in Poland and edge populations in France, finding that the edge populations had higher genotypic diversity. Further, even without genetic differences, peripheral populations can still be conservation priorities. For example, Lammi et al. (2001) studied *Lychnis Viscaria* at the edge and center of its range, finding low genetic variation within the edge populations but equal in viability to center range populations. Any viable populations could be vital to species survival during climate change.

1.9   Study System

1.9.1   *Eleocharis geniculata* **life history**

Eleocharis geniculata ((L.) Roem and Schult)) is an annual that grows in dense tufts (González-Elizondo and Reznicek 1996) and requires open areas with low competition from other plant species (Bowles 2010). *Eleocharis geniculata* is a late-senescing species that becomes mature in late summer or early fall, producing bisexual flowers and then diagnostically black achenes (Svenson 1929, Bowles 2010, Voss and Reznicek 2010). Being an annual plant, *E. geniculata* is dependent on its achenes surviving in the seedbank to maintain local populations (COSEWIC 2009). The achenes may remain dormant in the seedbank for several years, though the exact duration of dormancy is unknown. The dispersal of achenes controls the distribution of the species (COSEWIC 2009). The achenes have limited means of dispersal; however, water fluctuations may spread achenes around wetlands (COSEWIC 2009). Plants grow and create achenes only during ideal conditions, and suitable habitat can vary greatly depending on the water fluctuations from year to year (Bowles 2010). The suitable water depth conditions for germination are also currently unknown (COSEWIC 2009). There is a clear knowledge gap in our understanding of the germination needs and reproductive biology of *E. geniculata*.

1.9.2   **North American distribution**

Eleocharis geniculata is widely distributed throughout southern North America (Figure 1.1; COSEWIC 2009). It is located around the Great Lakes region of Michigan, southern Ontario, and Indiana continuing south to Florida, West to Texas and California and north again to British Columbia (Crow and Hellquist 2000). At the northern extent of its North American range, Canada contains two genetically distinct populations of *E. geniculata,* one in British Columbia and one in Ontario (COSEWIC 2009).

The Ontario population is restricted to sandy or muddy soils along ponds, ephemeral pools and other wet areas (Ward and Leigh 1975, Crow and Hellquist 2000, COSEWIC 2009, Voss and Reznicek 2010). Although not considered threatened across most of its North American range, the peripheral population in Ontario is classified as endangered under the Canadian Species at Risk Act (S.C., 2002) and the Ontario Endangered Species Act (S.O., 2007). It is considered a conservation priority (COSEWIC 2009). The population in Ontario has adapted to different

environmental conditions from individuals at the center of its range (COSEWIC 2009). One example is differences in temperature during the day throughout *E. geniculata*'s growing season. At the southern end of the North American range in Key West Florida, the average temperature during the day is 29°C compared to the average temperature of 19°C of Long Point, Ontario at the North end of the range (National Oceanic and Atmospheric Administration N/D). Therefore, the Ontario population could be vital to the long-term survival of the species, particularly as the climate continues to change. Importantly, there is little known about this species' habitat requirements in Ontario (COSEWIC 2009).

In 2007, a search was conducted in the three regions where *E. geniculata* was reported to occur in the past (Figure 1.2; COSEWIC 2009). These include Cedar Springs (300-500 individuals) and the Long Point National Wildlife Area (1000-2000 individuals), where extant populations were observed (COSEWIC 2009). The third patch of *E. geniculata* in Ontario was in Rondeau Provincial Park but has not been seen since 1934 (Bowles 2010). However, the park had not been thoroughly searched since 2007 (COSEWIC 2009), and the 2007 survey did locate *E. geniculata* in nearby Cedar Springs, suggesting that Rondeau Provincial Park warranted a thorough survey.
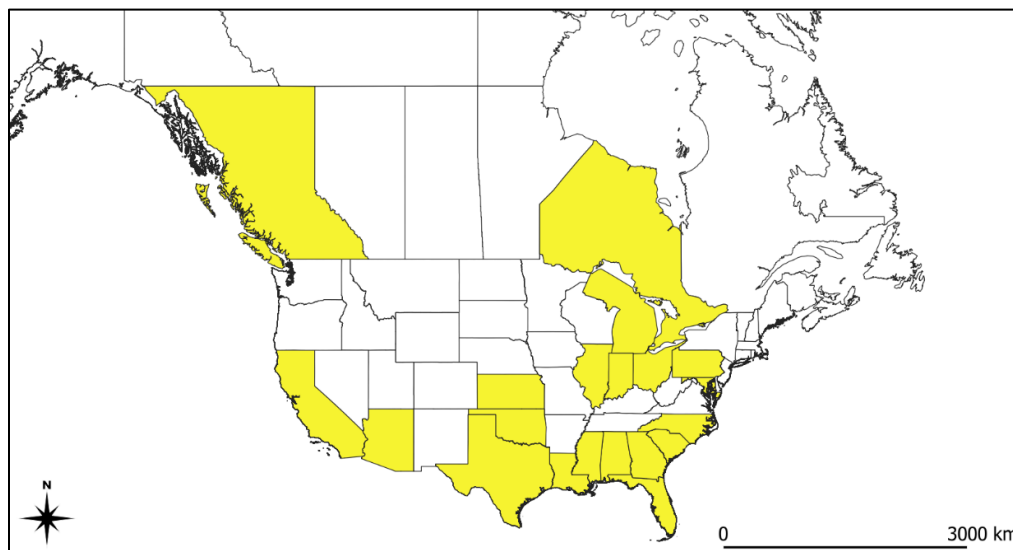
Figure 1.1. Map of *Eleocharis geniculata*'s North American distribution. The yellow fill shows North American occurrence of *E. geniculata* at the state or provincial level. Adapted from the United States Department of Agriculture Plants Database, no date. Map created using QGIS (QGIS Development Team, 2020).

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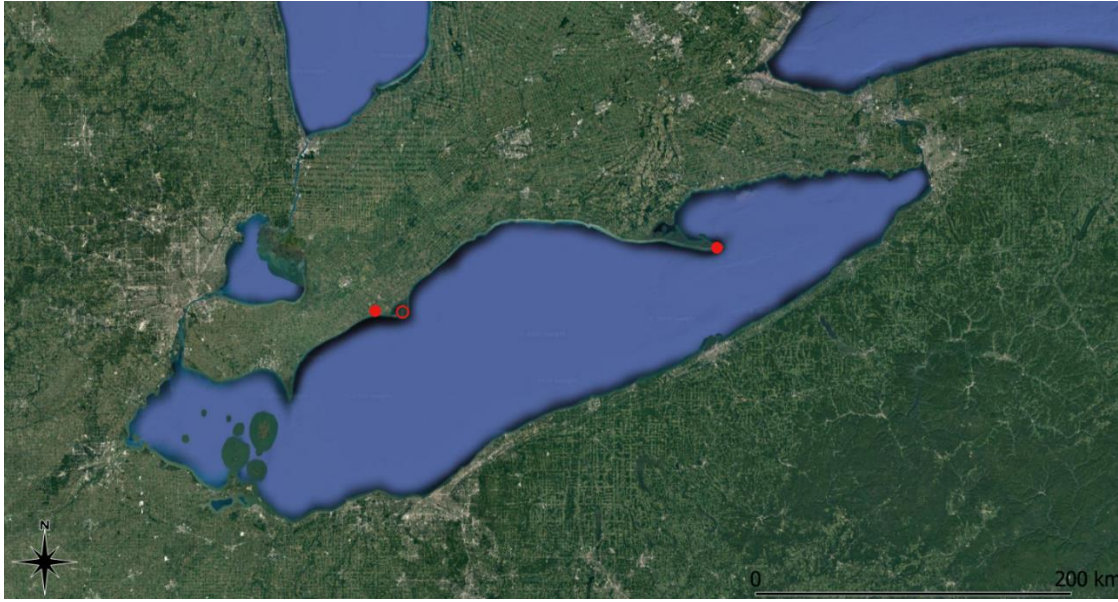
Figure 1.2. Location of *Eleocharis geniculata* Great Lakes Plains Population in Ontario. The solid circles represent sites where it was last observed during systematic searches in 2007 and the hollow circle represents the population in Rondeau Provincial Park that is believed to be extirpated (Bowles 2010). Map created using QGIS (QGIS Development Team, 2020).

1.10 Threats

Numerous threats combine to endanger *E. geniculata* in Ontario. Climate change constitutes a direct threat by changing precipitation and temperature patterns, which alters lake levels in Lake Erie (Gronewold et al. 2013). This affects the amount of habitat available for *E. geniculata*, as well as *E. geniculata*'s phenology and annual life history strategy. However, climate change is also a "threat multiplier". For example, climate change can intensify the effects of habitat loss (Segan et al. 2016), species invasions (Chapman et al. 2016), or alter fire regimes (Weber and Flannigan 1997, Young et al. 2017). Human activities can further modify wetland ecosystems by changing water quality, which can also encourage the spread of alien invasive species (Ehrenfeld and Schneider 1991).

Alien invasive species are opportunistic (Kercher and Zedler 2004), often out-competing native flora in disturbed habitat (Jose et al. 2013). In the case of *E. geniculata*, invasion by European *Phragmites australis* ((Cav.) Trin. Ex Steud) is recognized as a significant threat (Bowles 2010). However, *E. geniculata* is far from alone. In Ontario, 25% of species at risk are identified as

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threatened by European *P. australis* (Bickerton 2015). Specifically, in the Long Point World Biosphere Reserve, where *E. geniculata* is believed to persist, European *P. australis* is recognized as displacing native vegetation in wetlands and moist shorelines (Wilcox et al. 2003) – critical *E. geniculata* habitat.

1.10.1 Invasive *Phragmites australis* biology and ecology

The genus *Phragmites* has one of the broadest ranges of any plant globally (Saltonstall and Meyerson 2016). Commonly, wetlands in temperate regions of the world are characterized by *Phragmites* (Saltonstall and Meyerson 2016). *Phragmites* can benefit wetland communities by providing services such as erosion control (Rooth and Stevenson 2000), wildlife habitat (Kiviat 2019) and water filtration (Yuckin and Rooney 2019). A native lineage has inhabited North American wetlands for thousands of years (Chambers et al. 1999). However, a lineage native to Europe has invaded North America where it is having detrimental effects on wetlands (Saltonstall 2002).

Invasive European *P. australis* (hereafter, *P. australis*) is believed to have invaded North America in the 19th century (Lelong et al. 2007). It became well established first along coastal marshes (Philipp and Field 2005, Chambers et al. 2008) in southwestern Nova Scotia (Catling and Mitrow 2011) and it is unknown how it was transported to Canada (Government of Ontario, 2019). Using natural corridors such as drainage ditches along highways and rivers (Lelong et al. 2007, Jodoin et al. 2008), *P. australis* spread into native wetlands along the Great Lakes (Wilcox et al. 2003, Tulbure et al. 2007, Jodoin et al. 2008, Brisson et al. 2010). In 1910, the first known population of *P. australis* was located in Nova Scotia (Catling and Mitrow 2011). By 1948 the first invasive population was found in Ontario and by 2010, *P. australis* had spread throughout southern Ontario, Southern Quebec, southern Manitoba and British Columbia (Catling and Mitrow 2011). Where it has already invaded, it is predicted to continue replacing native wetland species (Jung et al. 2017).

1.10.2 Invasive *Phragmites australis* reproduction

Phragmites australis has been so successful at invading North American wetlands because it has multiple ways to reproduce (Albert et al. 2015). *Phragmites australis* can grow and spread by seeds, stolons or rhizomes (Mal and Narine 2004), allowing it to invade new areas rapidly

(Meyerson et al. 2000, Ailstock et al. 2001). The primary method for long-distance dispersal is sexual reproduction (Albert et al. 2015). Inflorescences can contain thousands of seeds, which are wind-dispersed (Saltonstall et al. 2010). For short distance, asexual reproduction via stolons and rhizomes dominates. Stolons are vertical shoots that spread horizontally along the soil surface and from which grow new aerial shoots capable of photosynthesis and sexual reproduction (Brisson et al. 2010). Rhizomes grow underground, with aerial shoots emerging from nodes beneath the soil (Ailstock et al. 2001). Rhizomes and stolons aid in resource acquisition and transfer nutrients via clonal subsidy (Amsberry et al. 2000). This allows *P. australis* to initially invade ideal habitat from seeds and then spread vegetatively into less ideal conditions, where growth is supported by nutrients and sugars shared over the rhizome network (Amsberry et al. 2000).

1.10.3 Habitat disruption

The establishment of *P. australis* in the Long Point National Wildlife Area occurred rapidly in the late 1990s (Wilcox et al. 2003). One of the main plant community types that were outcompeted by *P. australis* during this time was marsh meadow (Wilcox et al. 2003): the habitat preferred by *E. geniculata*. As *P. australis* invades a community, it causes the reduction of air and soil temperatures and the amount of light that reaches the seedbank (Meyerson et al. 2000). This occurs because of the tall and dense growth form of *P. australis* which intercepts most incoming light and reduces soil temperatures (Meyerson et al. 2000). The reduced light levels prevent germination and survival of native species (Meyerson et al. 2000), likely including the much smaller annual *E. geniculata*. Further, *P. australis* is considered highly competitive when compared to native plants. For example, when comparing the invasive *P. australis* to the native *P. australis*, the invasive subspecies is 51% more efficient at photosynthetic processes (Mozdzer and Zieman 2010). Although the photosynthetic efficiency of *E. geniculata* is unknown, it is unlikely to be competitive with *P. australis*.

1.11 Thesis Scope

In chapter 2, I seek to fill the knowledge gaps outlined in the government response statement for *E. geniculata* (MNRF 2019) and the government recovery strategy (Bowles 2010). I intend to publish this chapter as a manuscript in the Canadian Field Naturalist or equivalent journal. The

objective of this chapter is to: 1) search critical habitat, locate and census located patches; 2) characterize the habitat requirements of *E. geniculata* by quantifying biotic and abiotic site characteristics at extant and recently occupied locations and; 3) characterize the threat and map the presence of *P. australis* adjacent to any *E. geniculata* patches to evaluate the risk that this invasion poses to remaining *E. geniculata*.

Achieving these thesis objectives is a necessary precursor to the successful recovery of *E. geniculata* in Ontario, at the northern extreme of its range. Conservation of this northern peripheral population may be essential to the conservation of the species' genetic diversity and adaptive capacity (Safriel et al. 1994). This is crucial in this period of environmental uncertainty where biodiversity is threatened by biological invasions, land use changes and climate change simultaneously (Bellard et al. 2012).

In the concluding chapter, I suggest future work, including testing different methods for controlling *P. australis* in areas with species at risk and continued monitoring of *E. geniculata*. I also discuss the significance of this research for maintaining biodiversity and the importance of protecting species at risk.

2 *Eleocharis geniculata* critical habitat surveys, habitat and threat characterization

2.1 Introduction

Climate change is a major threat to biodiversity (Thomas et al. 2004). In response, many species are shifting their distributions poleward from their historic boundaries (Parmesan 2006). This makes the conservation of populations across the entire range of a species vital (Gapare et al. 2005, Leppig and White 2006, Bateman et al. 2015). Peripheral populations of species may have novel evolutionary potential from adapting to different abiotic and biotic pressures than those faced by individuals at the center of its range (Leppig and White 2006). These peripheral populations could be genetically distinct and potentially better suited to future climate conditions, making them high conservation priorities (Leppig and White 2006). An example of this is *Eleocharis geniculata* ((L.) Roem and Schult)), a wetland plant with a peripheral population in Ontario, Canada that is endangered and in need of conservation, though the species is not classified as at risk in its core range (NatureServe 2017).

2.2 *Eleocharis geniculata* distribution

Eleocharis geniculata is widely distributed throughout southern North America (Figure 1.1; COSEWIC 2009). It is located around the Great Lakes region of Michigan, southern Ontario, and Indiana continuing south to Florida, west to Texas and California and north again to British Columbia (Crow and Hellquist 2000). The Canadian populations are the northern most in North America.

In Canada, *E. geniculata* exists as two genetically distinct populations, one in British Columbia and one in Ontario (COSEWIC 2009). The British Columbia population, known as the Southern Mountain population, is located on a sand spit in Osoyoos Lake (COSEWIC 2009). The Ontario population, known as the Great Lakes Plains population, is thought to be separated spatially among three locations: Cedar Springs, Rondeau Provincial Park and Long Point National Wildlife Area (Figure 1.2. COSEWIC 2009).

Despite its status as endangered in Ontario, there is no sustained monitoring of *E. geniculata*. Prior to my work, 2007 was the last targeted search of all historically known locations of *E. geniculata* (COSEWIC 2009). Rough population estimates in 2007 predicted 300-500 individuals were present in Cedar Springs and 1000-2000 individuals were present in Long Point National Wildlife Area (COSEWIC 2009). However, *E. geniculata* has not been reported from Rondeau Provincial Park since 1934 and it may now be extirpated (COSEWIC 2009). In 2014, anecdotal sightings of *E. geniculata* occurred the Long Point National Wildlife Area (Michael Oldham, pers. comm. 2017). The government response statement (MNRF 2019) and the Ontario government recovery strategy (Bowles 2010) states that a systematic survey is needed to locate areas of conservation importance and to characterize *E. geniculata* habitat.

In addition to questions regarding its current distribution and the urgent need for a census, the Ontario government recovery strategy identifies key knowledge gaps that present obstacles to conservation and recovery of *E. geniculata* in Ontario (Bowles 2010). These knowledge gaps pertain to: 1) *E. geniculata*'s population dynamics, 2) its habitat requirements and 3) the threat presented by invasive species, particularly European Common Reed (*Phragmites australis* ((Cav.) Trin. Ex Steud) (Bowles 2010).

1) Population dynamics

Eleocharis geniculata is a late season annual that grows in dense tufts (González-Elizondo and Reznicek 1996). Being an annual plant, *E. geniculata* is dependent on its achenes surviving in the seedbank over winter to maintain local populations (COSEWIC 2009). The achenes may remain dormant in the seedbank for several years, and their dispersal controls the distribution of the species (COSEWIC 2009). The length of time that *E. geniculata* achenes persist in the seedbank is unknown (COSEWIC 2009). Little is known about how many achenes an adult can produce, how achenes disperse, or what their germination requirements are. Further, little is known about interannual variation in their population size tied to recruitment from the seedbank. Regular, long term monitoring is needed to establish population trends (Bowles 2010) and identify habitat requirements.

2) Habitat requirements

In Ontario, the population is restricted to sandy or muddy soils along ponds, ephemeral pools and other wet areas (Ward and Leigh 1975, Crow and Hellquist 2000, Voss and Reznicek 2010). *Eleocharis geniculata* likely requires open areas with low competition from other plant species, because it is a short (< 20 cm) plant that grows late in the season (Bowles 2010). *Eleocharis geniculata* individuals grow and create achenes only during ideal conditions and suitable habitat can vary greatly depending on water-level fluctuations from year to year (Bowles 2010). It also likely requires water drawdown to encourage its germination from the seedbank (Van Leeuwen et al. 2014).

Though there are anecdotal reports about *E. geniculata*'s habitat requirements and tolerance for competition, there has never been a study to quantify this. Currently, the ideal water depth for the Great Lakes Plains population of *E. geniculata's* germination and its tolerance to seasonal water fluctuations is unknown. Additionally, we do not know the range of soil quality and temperature that *E. geniculata* can tolerate. The level of aboveground competition for light endured by *E. geniculata* is also unknown.

3) Threats

2.2.1 Invasive *Phragmites australis*

Unfortunately, the wet, sandy or muddy soils thought to be preferred by *E. geniculata* also support the growth of invasive *P. australis*. This European lineage of *P. australis*, hereafter simply *P. australis*, invaded North America (Saltonstall 2002), where it is disrupting nutrient cycles (Bernal et al. 2017), degrading ecological integrity (Meyerson et al. 2000, Wilcox et al. 2003) and compromising biodiversity (Meyerson et al. 2000, Rojas and Zedler 2015). Important factors in *P. australis'* ability to outcompete native wetland plants include its rapid growth, extended growing season (Juneau and Tarasoff 2013), litter accumulation (Warren et al. 2001), and light interception (Hirtreiter and Potts 2012). *Phragmites australis* threatens native fauna and flora, like *E. geniculata*, in part because it grows so densely (Ailstock et al. 2001) and reaches canopy heights up to 6 m tall (Meyerson et al. 2000, Mal and Narine 2004).

As *P. australis* invades a community it causes the reduction of air and soil temperatures and the amount of light that reaches the seedbank (Meyerson et al. 2000). The reduced light levels prevent gemination and establishment of native species (Meyerson et al. 2000), likely including the much smaller annual *E. geniculata*. Although the actual effects of competition between *P. australis* and *E. geniculata* have never been studied, research in the USA and Canada underscore the tendency of *P. australis* to competitively exclude other wetland species (Wilcox et al. 2003, Minchinton et al. 2006). For example, a review by Bickerton (2015) concluded that 25% of Ontario's 217 species at risk were threatened by *P. australis* invasion. Further, vascular plants were found to be the most affected taxonomic group within this review (Bickerton 2015). Consequently, the government response statement for the Great Lakes Plains population of *E. geniculata* identifies *P. australis* invasion as one of the primary threats to *E. geniculata*'s persistence in Ontario (MNRF 2019).

In Long Point, the 2007 survey for *E. geniculata* found a smaller number of *E. geniculata* than the 1988 survey (Bowles 2010). The survey also revealed that *P. australis* had expanded into potential *E. geniculata* habitat (Bowles 2010). It is unknown to what extent *P. australis* has continued encroaching on *E. geniculata* since 2007, or if *E. geniculata* is capable of coexisting with *P. australis*.

2.2.2   Habitat loss

The threat of invasive species is compounded by habitat loss. By 2002, Southern Ontario had converted 72% of its pre-settlement wetlands into alternate land uses (Ducks Unlimited Canada 2010). Long Point contains >80% of the remaining intact costal marsh habitat on the north shore of Lake Erie (Ball et al. 2003). The loss of intact coastal marsh habitat for *E. geniculata* in the region is compounded by the *P. australis* invasion of *E. geniculata*'s critical habitat (COSEWIC 2009), such that there is very limited suitable habitat remaining.

Habitat loss and the invasion of *P. australis* are well documented, however the direct effects on *E. geniculata*'s abundance are unknown. In addition, the distribution of actual available habitat for *E. geniculata* within the identified critical habitat area of *E. geniculata* is unknown. Determining the distribution of available habitat is important because *E. geniculata* habitat restoration and conservation efforts cannot begin until the habitat is identified.

2.2.3 Climate change

Threats to *E. geniculata* from habitat loss are likely to be exacerbated by climate change (Monzón et al. 2011). Climate change is affecting temperatures (Alley et al. 2007), hydrological cycles (Ferrati et al. 2005), and generally causing more extreme and unpredictable weather patterns (Chen et al. 2011, Bellard et al. 2012). Climate shifts will lead to shifts in suitable habitat for many species (Monzón et al. 2011). Movement of species towards the poles has already been documented (Parmesan 2006). Habitat shifts can threaten species at risk more, as suitable habitat shifts outside of protected areas (Monzón et al. 2011). The ability of the Ontario population of *E. geniculata* to adapt and withstand a larger range of environmental variables as a result of climate change is unknown. Climate change could leave *E. geniculata* without suitable habitat in Ontario, particularly in combination with the threat of expanding *P. australis,* compounded by historic and ongoing wetland loss.

2.3 Objectives

My goal is to aid in the recovery and protection of the Ontario population of *E. geniculata* by filling some of these knowledge gaps. To address the gap around the current distribution and population census of *E. geniculata* in Ontario, I searched all locations where *E. geniculata* was reported to occur historically and all seemingly suitable habitat within the area designated as *E. geniculata* habitat under section 24.1.1 of Ontario Regulation 242/0814 (Endangered Species Act 2007) to provide information on the current location of patches of *E. geniculata* and highlight areas of conservation importance or in need of restoration. Once I located a patch, I mapped the area, and censused the patch to estimate the population size. To address knowledge gaps around population dynamics, particularly the number of achenes produced by an adult, I enumerated the number of adult stems bearing fruit and the number of achenes per fruit-bearing stem to estimate the contribution of achenes to the seedbank each year. To address knowledge gaps about the habitat requirements of *E. geniculata* and, to better define critical habitat for the Great Lakes Plains population, wherever I located *E. geniculata*, I also recorded co-occurring species, water levels, soil quality, and light availability. I supplemented the soil nutrient analysis by analyzing stable isotopes and C and N concentration in *E. geniculata* aboveground and belowground tissues to determine nitrogen source, nitrogen limitation, and to provide information about water use efficiency. Finally, to evaluate the magnitude of the threat to the Great Lakes Plains

population of *E. geniculata* presented by *P. australis*, I mapped the distribution of any *P. australis* within the wetland growing within 100 m of *E. geniculata* patches. I also measured water use efficiency and leaf-level carbon assimilation to compare the two species to quantify the difference in competitive capacity between them. Addressing these knowledge gaps was identified as a high priority in the government response statement for *E. geniculata* (MNRF 2019) and the government recovery strategy (Bowles 2010). This reflects the urgency with which conservation and recovery of this key peripheral population hinges on better understanding its current distribution, population dynamics, habitat needs, and relationship to *P. australis*.

2.4 Methods

This research was carried out under permit AY-B-006 under the Ontario Endangered Species Act and permit SARA-OR-2017-0382 under Section 73 of the Canadian Species at Risk Act.

2.4.1   Study area

I conducted surveys for *E. geniculata* in 2017 and 2018. Surveys for *E. geniculata* were distributed across three locations: Cedar Springs, Rondeau Provincial Park, and the Long Point National Wildlife Area. All are located in southwestern Ontario (Figure 1.2).

Cedar Springs (42.2805° N, 82.0313° W) is a township located in the municipality of Chatham-Kent in southern Ontario. It consists of sandy soils suitable for fruit farms. Permission was a limiting factor for searching in Cedar Springs. The critical habitat in this area consisted of private property, farmland, gravel quarries, and manicured lawns.

Rondeau Provincial Park (42.3174° N, 81.8471° W), established in 1984, (Ministry of the Environment, Conservation and Parks (MECP) 2020) is located on a spit along Lake Erie. It is 3254 ha of forest, wetlands, dunes, natural grass prairies and beach (MECP 2020). Over 300 species of migratory birds use this protected area as a stopover during migration (MECP 2020).

Long Point National Wildlife Area (42.5471° N, 80.1540° W) is located at the eastern tip of the Long Point sand spit in Lake Erie. Established in 1978, it protects 3284 ha of wetlands, dunes, and wildlife (Environment and Climate Change Canada (ECCC) 2017). Long Point is also a vital habitat that supports over 300 species of migratory birds (ECCC 2017).

2.4.2 Critical habitat surveys

I searched historical points, the location of historical *E. geniculata* individuals, obtained from Environment Canada (2015) and National History Information Center (NHIC 2017). I expanded the search into nearby wet areas to cover all candidate habitat. Using Google Earth satellite imagery, I located all wet areas within 3 km (for Cedar Springs (Figure 2.1, Google Earth V 7.3.2.5776 2019a)) or within 1 km (for Rondeau Provincial Park (Figure 2.2, Google Earth V 7.3.2.5776 2019b) and Long Point National Wildlife Area (Figure 2.3, Google Earth V 7.3.2.5776 2019c)) of the historical points depending on the location.

In late August to early September, when *E. geniculata* was growing, I walked the perimeter of each potential habitat location to search for *E. geniculata*. To ensure minimal disruption to the habitat, the search team consisted of two individuals, and we only walked off the trail to search a good quality habitat. I identified any species at risk viewed during surveys. I recorded the dominant plant species, water depth, soil type, presence or absence of *P. australis* and I photographed each survey location.

Survey effort varied with the complexity of the terrain. Every suitable wet area (sandy, full sun exposure, minimal competition from co-occurring species), I thoroughly searched, checking every *Eleocharis* species I located for the diagnostic black achenes of *E. geniculata*. In heavily vegetation wet areas with organic sediment, I did not search exhaustively, as this habitat is considered ill suited for *E. geniculata*. Regardless of *in situ* suitability, at every candidate habitat site that I surveyed I recorded the dominant vegetation cover and the water depth.

2.4.2.1 *Cedar Springs*

For all sites designated as candidate habitat in this area, I approached property owners for access to their property. If access was not permitted, the potential habitat was not searched. In total, I searched 25 potential habitat sites between June 14, 2017, and August 23, 2017 (Figure 2.1). Notably, my survey dates were early in the season, and so I did not expect to see adult *E. geniculata*, but I could confirm if habitat remained suitable for *E. geniculata* and resurvey any potentially suitable habitat later in the season.

2.4.2.2 *Rondeau Provincial Park*

I searched all candidate habitat locations in Rondeau Provincial Park on September 10 and 11, 2018 at a time when *E. geniculata* would be evident if it were present (Figure 2.2).

2.4.2.3 *Long Point National Wildlife Area*

From June 20 to June 23th, 2017, I searched many of the candidate habitat areas within the five 1 km x 1 km grids of critical *E. geniculata* habitat designated in the National Wildlife Area (Figure 2.3). Unfortunately, due to time constraints, I was only able to search 41 of the 83 wet areas within the potential critical habitat area. However, on September 18 and September 19, I conducted another targeted search of the 29 historical locations. Again, due to time constraints, not all of the critical habitat could be searched, so I decided to focus on the areas *E. geniculata* was most likely to occur. On September 26 to October 3, 2018, I was able to search all of the candidate habitat areas. It is important to note that Birds Canada has treated some critical habitat wetlands with a glyphosate-based herbicide to control *P. australis*. The treatment occurred during both fall 2017 and 2018, at time when adult *E. geniculata* would exist.

Figure 2.1. Cedar Springs critical habitat search area (42.2805° N, 82.0313° W; n = 25). The historical point of *E. geniculata* was located within this grid. Every wet area or pond was searched that fell within the 3 km^2 grid. Map created using QGIS (QGIS Development Team, 2020, Google Earth V 7.3.2.5776 2019a).

Figure 2.2. Rondeau Provincial Park critical habitat search area (42.3174° N, 81.8471° W; n = 21). Searches occurred at every wet area or pond within the 1 km^2 grid squares and were limited to wetlands with sandy soils. Map created using QGIS (QGIS Development Team, 2020, Google Earth V 7.3.2.5776 2019b). It should be noted that due to high water levels, many wet areas were flooded, preventing access.

Figure 2.3. Long Point National Wildlife Area critical habitat search area (42.5471° N, 80.1540° W; n = 83). The 1 km^2 grid squares contain the historically known points of *Eleocharis geniculata* and all wet areas that were searched. Map created using QGIS (QGIS Development Team, 2020, Google Earth V 7.3.2.5776 2019c).

2.4.3 Population estimates

Wherever I located a patch of *E. geniculata*, I measured the size of the patch and censused the population size. To estimate the population size of the *E. geniculata* patches, I employed two different methods depending on patch size and density. Patches were determined to be "large" (greater than 100 individuals) or "small" (less than 100 individuals). To estimate the extent of small patches, I used a metre stick to measure the entire area of the patch or sub-patch. I considered a sub-patch to be patches of *E. geniculata* that are separated from each other by other vegetation. For patches I deemed "large," I paced the area using a high- precision GPS to digitally track the perimeter and then calculate its extent. For small patches, I censused the population by counting each individual within the patch. For large patches, my approach differed based on population density. In a densely populated area (i.e. $\geq$ 1000 individuals per m^2), I randomly placed five 5 cm^2 quadrats, whereas, in a sparse population area, I randomly placed ten 0.25 m^2 quadrats. Within each quadrat, I counted all individuals and then I extrapolated to the measured patch extent to estimate the population size. To ensure population size estimates were as accurate as possible, I located the base of each fascicle, counting one fascicle as one individual.

2.4.4 Habitat characterization

Because *E. geniculata* is a late-growing species, to characterize the co-occurring species and hydrologic variability of sites where *E. geniculata* could grow, I had to take measurements at sites where *E. geniculata* had previously been encountered. I was not certain that it would grow in the areas I characterized until much of the data had already been collected in 2017. For this reason, I focused on two locations where *E. geniculata* was observed. One of these locations was an incidental report of occurrence by Ontario Botanist Michael Oldham in 2014 at a site named Pond 2 in the Long Point National Wildlife Area (pers. Comm. 2017). The other site, Pond 9, was chosen for its easy accessibility and because it contained historical occurrences from 2007. In 2018, I conducted habitat characterizations at Pond 2 and Pond 9 in the Long Point National Wildlife Area. I also carried out habitat characterization activities in any sites where I found *E. geniculata* growing in fall of 2017.

2.4.5 Co-occurring species and phenology

In 2018, at Pond 2, Pond 9 and at any extant patch of *E. geniculata* I detected in 2017, I comprehensively surveyed all plant species repeatedly throughout the growing season. Using 25 randomly placed 0.25 m² quadrats, I estimated the area covered by each species. I identified species in the field if possible; otherwise, samples were collected, pressed and identified in the lab. I also recorded their phenological phases. Following the Denny et al. (2014) protocol for recording phenological data, I recorded commonly monitored phenophases in grass and sedge species (i.e., initial growth, young leaves, first flower, pollen release, first fruits, ripe fruits, and seed-drop).

To visualize the patterns in co-occurrence data among the vegetation quadrats surveyed in any located patches of *E. geniculata*, I used NMS ordination, carried out in PC-ORD v. 7.08 (McCune and Mefford 2018). I first arcsine square-root transformed the vegetation cover data from all plots and then excluded any vegetation cover types not present in at least two of 25 surveyed quadrats. I calculated Sorenson's distance measure to characterize dissimilarity among the quadrats in my NMS. I identified the optimal number of dimensions by comparing the stress from 50 runs with real data and 50 runs with randomized data from 1 to 4 dimensional solutions, with each run comprising up to 200 iterations to reach the stability criterion of 0.00001. I then graphed the joint plot from the ordination where I superimposed vectors reflecting the strength and direction of correlation between the percent cover of each cover type and the NMS ordination axes, providing the cover type had an r^2 value > 0.15 with at least one of the axes.

2.4.6 Water levels

To determine the range of water levels experienced by *E. geniculata* patches, I used HOBO data loggers (HOBO pressure transducers with temperature compensation, Bourne, MA). In 2017, on July 25 until September 19, I installed a logger at Pond 2 and Pond 9. In 2018, on June 2 until September 28, I installed depth data loggers in the same locations as 2017, adding an additional logger, on June 1, close to any extant 2017 location. In both years, I also installed a barometer to inform barometric compensation for water depth determination (Figure 2.4).

In 2017, I programmed the loggers to take temperature and pressure measurements every hour and in 2018, every two hours. This is because in 2018, I deployed the loggers earlier and I

wanted to ensure that the data would not fill the internal storage and prevent recording. Using Onset HOBOware (version 3.7.16; Onset Computer Corporation, Bourne, MA), I averaged readings to obtain daily mean water depth. Additionally, I calculated the weekly amplitude of the pond near the *E. geniculata* located in 2017, Pond 2 and Pond 9. I included August and September only, because that is when *E. geniculata* germinates and grows. I also calculated the monthly amplitude at the three locations for the whole growing season.

Figure 2.4. The location of HOBO water depth data loggers and associated barometer in the Long Point National Wildlife Area (n = 4). The red 1 km^2 square is where the barometer was located. The two yellow 1 km^2 squares contain pond 2 and pond 9, which have historical points from the 2007 systematic survey. The orange 1 km^2 square is *E. geniculata* patch 1, which is an extant patch that I located in 2017. Map created using QGIS (QGIS Development Team, 2020, Google Earth V 7.3.2.5776 2019c).

2.4.7   Soil quality

2.4.7.1   *Conductivity, temperature and moisture*

In 2017, I used a temperature compensating soil conductivity probe (Hanna HI 98312 DiST 6 EC/TDS/Temperature Tester, Limena, Italy) and HHR Moisture Meter (Delta-T Devices, Cambridge, UK) to measure soil conductivity, temperature and moisture across any extant patch

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of *E. geniculata* I encountered. I took two measurements in every sub-patch to ensure good representation of spatial variation in conditions where *E. geniculata* was extant.

2.4.7.2 *Nutrients, moisture and texture*

In 2018, at each extant patch of *E. geniculata*, I collected three replicate soil cores measuring 10 cm in depth, using a 6.5 cm diameter stainless steel soil auger. The replicates were collected throughout the extant *E. geniculata* patches, to analyze the range of nutrient levels, soil moisture and texture. Soil was kept cool after collection and transported to the University of Guelph Agriculture and Food Laboratory for analysis of macro (total C, N, P, and K) and micro (Mg, Ca, Zn, Cu, Na, Fe) nutrients following standard methods. When cations were below the limit of quantification, I treated the value as ½ the limit of quantification when averaging across replicates. Phosphorous was extracted using sodium bicarbonate extraction (Olsen et al. 1954). For magnesium, potassium, calcium and sodium, ammonium acetate extraction method was used (Simard 1993). Ammonium and nitrate were total extractable amounts and were extracted into a 2 M KCl solution prior to spectrophotometric analysis on a Seal AQ2 discrete analyser (SEAL Analytical Inc., Mequon, Wisconsin) following standard methods (United States Environmental Protection Agency (USEPA) 1983; USEPA 1993). Zinc, copper and iron were extracted using the DTPA extraction method (Soltanpour and Schwab 1977). Manganese was extracted using phosphoric acid extraction (Reid 1998). Carbon was measured by combustion, using the Elementar Vario Macro Cube (Elementar Americas Inc., Mt. Laurel, NJ). Organic carbon was measured after three hours of combustion at 475 degrees C, and total carbon was measured after three hours of combustion at 950 degrees C. Inorganic carbon was quantified as the difference between total carbon and organic carbon.

2.5   Threat from *Phragmites australis*

2.5.1   Mapping

To assess the direct threat of *P. australis* to existing *E. geniculata* patches, I mapped the distribution of *P. australis* within about 100 m of any extant patches of *E. geniculata* using GPS technology (either SX Blue II GPS/GNSS, Geneq Inc., Montreal, PQ, Canada or GPS MAP 64s, Garmin International Inc., Kansas City, KA, USA, depending on equipment availability). Due to time constraints, I prioritized areas that were directly connected to any extant patch and

dedicated less time to searching for *P. australis* in more distant portions of the 100 m buffer that were, for example, on the other side of a sand dune. I processed the GPS points and created the 100 m buffers using QGIS (version 3.4.15, QGIS Development Team 2020).

2.5.2 Water use efficiency and carbon assimilation rate

Comparing water use efficiency and carbon assimilation by *P. australis* and *E. geniculata* will reveal the magnitude of competitive asymmetry between the two plant species. In 2018, I collected *E. geniculata* water use efficiency and carbon assimilation information using a CIRAS-3 Portable Photosynthesis System (PP Systems, Amesbury, MA). This infrared gas analysis system simultaneously measures differential H_2O and CO_2 gas concentrations with four non-dispersive infrared gas analyzers to calculate carbon assimilation and water use efficiency. The leaf cuvette is also equipped with an LED light to permit precise control of light intensity for the creation of carbon assimilation curves. I took ten measurements from randomly selected individual *E. geniculata* at any location where it was found. Also, in 2017, I collected measurements from *P. australis* in the Long Point area using the same true-differential infrared gas analysis system.

2.5.3 Light availability

It is known that *E. geniculata* requires open areas to grow, and this is believed to be a restriction due to its poor ability to compete for light. To characterize the light needs of *E. geniculata*, I measured the percent of insolation reaching the top of *E. geniculata* (i.e., light penetration) in 2018. These measurements were unfortunately taken on a cloudy day and time restrictions prevented options for alternative days. I then compared this light level to the levels of light penetration through a *P. australis* stand in the Long Point area (Robichaud 2016, unpublished data) and my carbon assimilation curves for *E. geniculata* (described above) to extrapolate the ability of *P. australis* to shade out *E. geniculata* if the two co-occurred. Light levels and percent light intercepted by the canopy of *P. australis* were measured using a Li-Cor PAR sensor system equipped with two Li-Cor 190R quantum sensors (Lincoln, NE). To measure light interception, one sensor was held at the top of the *P. australis* canopy while the second sensor was held at the top of the substrate. This provided instantaneous readings of PAR intensity at the top and bottom of the canopy and avoided issues with light variability. For *E. geniculata*, measurements were

taken at the top of individuals to measure light requirements. Readings were taken between 11:00 and 13:00 hours to ensure the angle of incidence was consistent and direct.

2.5.4   Stable isotopes

To investigate photosynthetic pathways, water use efficiency and nitrogen sources of *E. geniculata* and *P. australis*, I measured the stable isotopes of δ^{13}C and δ^{15}N. I collected two individuals of *E. geniculata* and used 24 individuals of *P. australis* that were previous collected by a lab member. I was able to divide the two *E. geniculata* individuals into five stem samples and four root samples. There were not enough roots to make five samples. Fry et al. (1992) provides details of the elemental analyzer isotope ratio mass spectrometry method of analysis that I used. Briefly, plant tissues were washed, chopped, dried, and ground to a homogenous powder using a ball mill. The homogenous powder underwent combustion conversion to gas through a 1108 Elemental Analyzer (Fisons Instruments) coupled to a Delta Plus XL (Thermo-Finnigan, Germany) continuous flow isotope ratio mass spectrometer. The δ^{13}C values were corrected to the primary reference scale of Vienna Pee Dee Belemnite, whereas the δ^{15}N was corrected against the primary reference scale of atmospheric air. Analyses were carried out by the Environmental Isotopes Laboratory at the University of Waterloo.

2.6   Results

2.6.1   Critical habitat surveys

2.6.1.1   *Cedar Springs*
All potential habitat comprised of private lawns, farmland or gravel mines. Fifty percent of the searched sites, including the site where *E. geniculata* was reported to occur in the Natural Heritage Information Center database, were invaded by *P. australis* (Table 2.1). Despite conducting my survey early in the growing season, I conclude that *E. geniculata* is likely extirpated from the Cedar Springs area due to the highly disturbed nature of the areas that I did search and the extensive spread of *P. australis*. Although, future searches on properties I was denied access to are recommended.

2.6.1.2   *Rondeau Provincial Park*
I was unable to locate any *E. geniculata* in Rondeau Provincial Park. Most areas of candidate habitat were heavily vegetated, and 29% were invaded by *P. australis* (Table 2.1). Only one site

possessed any *Eleocharis* spp. at all, and although I did not definitively identify it, I do know that it was not *E. geniculata* due to the rhizomatous growth form.

In 2017, during the September search of the 29 historically known locations, I located Patch 1 of *E. geniculata* (Table 2.1). Notably, of the 29 locations in Long Point National Wildlife Area where *E. geniculata* had been observed historically, 18 (62%) were invaded by *P. australis*. In 2018, I located Patch 1 again. Further, I located a new patch, Patch 2, in a separate wet area (Table 2.1). I searched the wet area that Patch 2 was located in 2017 but in June before adults would be visible. It is unknown if Patch 2 had adult individuals in 2017. Further, the wet area that Patch 2 was located in was a new occurrence with no historical records. Patch 1, however, was located in a wet area that does have a historical observation from 2007.

Table 2.1. Summary table of the number of sites searched in Cedar Springs, Long Point National Wildlife Area and Rondeau Provincial Park. For more detailed results, see Appendix (Table S1, Table S2, Table S3, Table S4, and Table S5).

Region	Number of sites searched	Number of sites invaded by *Phragmites australis*	Number of sites with non-*E. geniculata Eleocharis* spp.	Number of sites with *Eleocharis geniculata*
Cedar Springs	25	13	17	0
Long Point National Wildlife Area (2017)	41	22	27	1
Rondeau Provincial Park	21	6	1	0
Long Point National Wildlife Area (2018)	83	41	45	2

2.6.2   Population estimates

2.6.2.1   Patch 1

The patch of *E. geniculata* that I located in the Long Point National Wildlife Area in 2017 was divided into four sub-patches (Table 2.2). I call this Patch 1 for ease of reference. I enumerated

44 individuals in the patch, comprising 737 culms, with 712 seed heads. The average height of the *E. geniculata* in the patch was 9.2 cm ($\pm$ 0.75, error showing standard error). The estimated spatial extent of the population was about 6.3 m^2. This patch was located in a wetland that Birds Canada treated with herbicide to control *P. australis* in fall of 2017. It is possible that this treatment affected the number of individuals of *E. geniculata*. The number of achenes was not collected at this time.

In 2018, when I surveyed Patch 1 again, it had expanded to consist of 16 small sub-patches of *E. geniculata* (Table 2.2). The patch consisted of an estimated 532 individuals, comprising of an average of 51 culms ($\pm$ 5.66, n =21) and 49 seed heads per individual ($\pm$ 5.55, n = 21) with an average of 31 achenes per seed head ($\pm$ 2.56, n = 15). This yields an estimated 27,106 culms, 26,144 seed heads and 806,978 achenes. The average height of the *E. geniculata* was 6.75 cm ($\pm$ 0.57, n = 6). The estimated spatial coverage of the population was approximately 186 m^2.

2.6.2.2   *Patch 2*

In 2018, I located a second patch of *E. geniculata* (hereafter named Patch 2), situated on the south side of the Long Point National Wildlife Area peninsula (Table 2.2). This patch was divided into six sub-patches. Five of these sub-patches I considered "dense," with many tiny individuals grouped together and essentially no other plant species intermixed within the patches. The one other sub-patch of *E. geniculata* was sparse, with larger *E. geniculata* individuals growing among other species, including some low-density (<15% cover) *P. australis*.

There were an estimated 785,351 individuals summed across the five dense sub-patches of this patch. On average, there were 13 individuals in each 5 cm^2 quadrat. The average number of culms per individual *E. geniculata* was 11 ($\pm$ 0.99, n = 10), with an average of 10 seed heads ($\pm$ 0.85, n = 10) and an average of eight achenes per seed head ($\pm$ 2.22, n = 10), yielding an estimated 7,555,618 culms, 7,539,370 seed heads, and 60,314,957 achenes. The average height of *E. geniculata* was 2.46 cm ($\pm$ 0.29, n = 5). The dense sub-patches covered at total of 151 m^2.

There were an estimated 310,612 individuals in the sparse sub-patch of this patch. The average number of culms per individual was 11 ($\pm$ 0.89, n = 29), with an average number of nine seed heads ($\pm$ 0.89, n = 29) and an average of 18 achenes per seed head ($\pm$ 2.94, n = 10), yielding an estimated 3,106,120 culms, 2,795,508 seed heads and 51,568,018 achenes. The average height was 4.35 cm ($\pm$ 0.53, n = 15). The sparse sub-patch covered an estimated 5092 m^2.

Table 2.2. Population estimations of Patch 1 in 2017 and 2018 and Patch 2 in 2018. Patch 1 was estimated by counting the number of individuals in each patch. Patch 2 was estimated using quadrats and cross-multiplication with the area covered. Note that in 2017, the number of achenes per seed head was not collected (N/C).

Sub-patch name	Estimated *E. geniculata* individuals	Area (m^2)	Average number of culms per individual	Average number of seed heads per individual	Average number of achenes per seed head
Patch 1 (2017)	44	6.3	125	117	N/C
Patch 1 (2018)	532	186	51	49	31
Patch 2 (dense)	785,351	151	11	10	8
Patch 2 (sparse)	310,612	5092	11	9	18

2.6.3   Habitat Requirements

2.6.3.1   *Co-occurring species and phenology*

In 2018, I completed co-occurring species surveys and recorded phenology over the growing season. *Eleocharis geniculata* percent cover in Patch 1 was low (Table 2.3). Individuals covered an estimated 1 to 15% in Patch 1 (average 7.6 % ± 1.74, n = 10). *Eleocharis geniculata* percent cover in Patch 2 was much higher in the dense patches, ranging from 33 to 66% (average 46.4% ± 6.20, n = 5). The sparse sub-patch of Patch 2 was similar in *E. geniculata* cover to Patch 1 (0.25% to 25% cover, average 4.33% ± 2.34 n = 10), though the individuals comprised far fewer stems and had fewer seed heads than those I enumerated in Patch 1. Whereas the dense sub-patches in Patch 2 were dominated by *E. geniculata*, the sparse sub-patches in Patches 1 and 2 grew interspersed with other species. Combining the 25 quadrats in Patch 1 and Patch 2 together, *Equisetum variegatum* (Schleich.) has the strongest association with *E. geniculata* as it was identified in 18 of the 25 quadrats. In addition, *Juncus* spp. were located in 12 of 25 surveyed quadrats. Some species of *Juncus* still had identifiable features, whereas others did not at the time of survey. If the species could be identified it was considered separately from the grouping of *Juncus* spp. For example, *Juncus brevicaudatus* (Engelm.) was identified in eight quadrats.

The last strong association with *E. geniculata* was *Cyperus esculentus* L., identified in seven of the 25 quadrats.

Table 2.3. Average percent cover of co-occurring species for Patch 1, Patch 2 sparse and dense. These averages are based on species located within 0.25 m² quadrats. For more details, see Appendix (Table S6, Table S7, and Table S8).

Cover type	Patch 1 average percent cover (n = 10)	Standard error	Patch 2 sparse average percent cover (n = 10)	Standard error	Patch 2 dense average percent cover (n =5)	Standard error
Alisma spp.	4.75	1.88	-	-	-	-
Aster spp.	-	-	0.98	0.35	4.50	1.66
Bare sand	59.80	3.43	16.00	6.49	24.80	8.26
Bryophyta spp.	0.50	0.50	-	-	-	-
Carex spp.	0.60	0.50	3.53	1.97	1.20	0.97
Chara spp.	0.70	0.50	0.10	0.10	0.20	0.20
Cyperus esculentus	4.50	1.62	1.03	1.00	1.50	1.50
Eleocharis flavescens	0.63	0.33	-	-	-	-
Eleocharis geniculata	7.60	1.74	4.33	2.34	46.40	6.20
Eleocharis palustris	-	-	0.85	0.75	-	-
Eleocharis spp.	5.00	0.67	-	-	-	-
Equisetum variegatum	4.20	2.11	21.05	6.38	1.20	0.97
Helenium autumnale	0.50	0.50	-	-	-	-
Hypericum kalmianum	0.13	0.10	-	-	-	-
Juncus balticus/ J. arcticus	-	-	2.70	1.68	0.20	0.20
Juncus brevicaudatus	-	-	0.78	0.53	1.90	0.37
Juncus marginatus	2.25	1.51	-	-	-	-
Juncus spp.	7.15	3.27	1.68	1.48	-	-
General plant litter	-	-	1.35	0.54	-	-

Cover type	Patch 1 average percent cover (n = 10)	Standard error	Patch 2 sparse average percent cover (n = 10)	Standard error	Patch 2 dense average percent cover (n =5)	Standard error
Panicum spp.	-	-	0.10	0.10	0.70	0.49
Parnassia palustris	-	-	0.53	0.50	0.50	0.50
Phragmites australis	0.35	0.26	7.75	1.84	-	-
Phragmites australis litter	0.50	0.50	-	-	-	-
Populus deltoides	-	-	0.10	0.10	-	-
Potamageton spp.	0.25	0.25	-	-	-	-
Sagittaria spp.	2.25	0.87	-	-	-	-
Schoenoplectus pungens	0.03	0.03	-	-	-	-
Solidago spp.	0.25	0.25	-	-	-	-
Standing dead	-	-	3.70	2.56	-	-
Triglochin spp.	0.25	0.25	-	-	-	-
Typha spp.	1.00	0.55	0.03	0.03	-	-
Unknown spp.	0.05	0.03	0.05	0.03	-	-
Utricularia vulgaris	-	-	0.53	0.50	-	-
Water	-	-	28.60	8.98	18.20	12.30

I determined that a two-dimensional NMS ordination solution was best (Figure 2.5). The final solution had a stress of 12.72848, with an instability < 0.00001 after 65 iterations. The cumulative variance in dissimilarity among quadrats explained by this solution was 89.1%: 62.1% on NMS axis 1 and 27.0% on NMS axis 2. A visual interpretation of the ordination reveals that the quadrats from patch 1 were relatively tightly clustered with lower axis 1 scores. The quadrats from patch 2 were more dissimilar, though they tended to have higher axis 1 scores. The patch 2 quadrats were segregated into the dense and sparse sub-patches, with the sparse quadrats having higher scores on axis 2. Examining the joint plot (Figure 2.6), the percent cover of *E. geniculata* (axis 2: r = -0.656, r2 = 0.431) was greatest in the patch 2 dense sub-

patch. It was also inversely correlated with the percent cover of *E. palustris* (axis 2: r = 0.640, r2 = 0.410)), *Populus deltoides* (axis 2: r = 0.620, r2 = 0. 384), and *P. australis* (axis 2: r = 0.512, r2 = 0. 262).
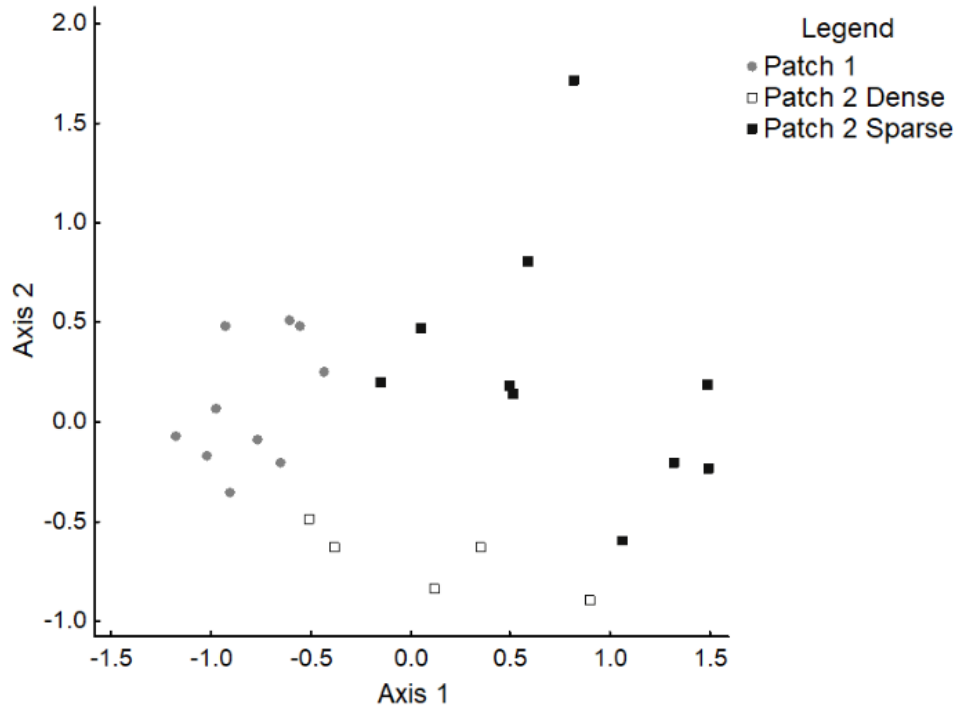
Figure 2.5. NMS ordination of patches. The first axis explains 62.1% if variance whereas the second axis explains 27.0%, resulting in a cumulative of 89.1% variance explained.
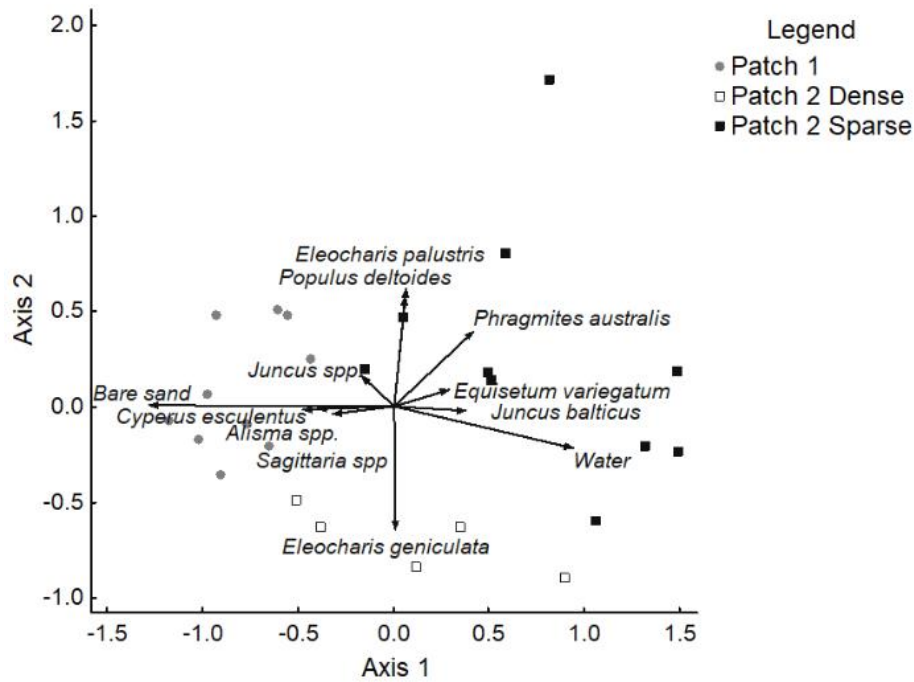
Figure 2.6. NMS ordination of co-occurring species. Patch 1 and Patch 2 sparse show the most difference, with Patch 1 associated with bare sand and Patch 2 sparse associated with *P. australis* and water. Patch 2 dense had higher percent cover of *E. geniculata* and is inversely correlated with *Populus deltoides*, *P. australis*, and *E. palustris*.

2.6.3.2   *Phenology*

In 2018, I surveyed and recorded the life stage of all species surrounding the historic patches in Ponds 2 and 9 as well as the extant Patch 1 (Table 2.4). Early indicators of potentially suitable habitat include *Juncus* spp.*, Triglocan* spp.*, Sorghastrum nutans* and *Carex buxbaumii*. Late season indicators include *Potamageton* spp. and *Calamagrostis canadensis*.

Table 2.4. Summary of co-occurring species phenological phases. These surveys occurred in 2018 at two historically known locations (Pond 2 and Pond 9) and at *Eleocharis geniculata* Patch 1. In this table I selected the phase that was seen the most in all survey locations or the phase that was most developed if different phases were seen in equal amounts across the survey locations. Y= young leaves, I= increasing leaf size, L=leaves, FB= flower/flower bud, O= open flower, F= fruit, R= ripe fruits, RD= recent seed/ fruit drop, U= unripe cones, S= senescing. For more details, see Appendix (Table S9, Table S10, and Table S11).

Species	Date of survey		
	28-Jun	05-Sep	28-Sep
Artemisia biennis	L	L	-
Aster spp.	-	-	O
Calamagrostis canadensis	-	-	RD
Calopogon tuberosus	O	O	-
Carex aurea	R	R	R
Carex buxbaumii	F	F	-
Carex pellita/Carex lasiocarpa	F	F	-
Castilleja coccinea	FB	FB	-
Dulichium arundinaceum	L	L	-
Eleocharis palustris	F	F	-
Equisetum spp.	FB	FB	F
Eupatorium perfoliatum	L	L	O
Fragaria virginiana	L	L	-
Gentianopsis crinite	-	-	O
Helenium autumnale	-	-	O
Hypericum perforatum	I	I	F
Iris spp.	L	L	-
Juncus spp.	FB	FB	RD
Juniperus horizontalis	L	L	L
Lathryus palustris	O	O	-
Liatris cylindracea	FB	FB	-

Species	Date of survey		
	28-Jun	05-Sep	28-Sep
Lobelia spp.	-	-	O
Lysimachia thyrsiflora	L	L	S
Medicago lupulina	O	O	-
Melilotus albus	FB	FB	-
Muhly spp.	-	-	F
Panicum flexile	FB	FB	F
Parnassia palustris	-	-	O
Phragmites australis	FB	FB	R
Poa spp.	FB	FB	-
Polygonum spp.	-	-	OF
Polygonum/Potamageton spp.	L	L	-
Pontederia cordata	FB	FB	S
Potamageton spp.	-	-	RD
Rosa palustris	L	L	R
Sagittaria spp.	-	-	S
Schenoplectus spp.	FB	FB	
Schoenoplectus acutus	-	-	S
Schoenoplectus pungens	O	O	S
Scirpus microcarpus	F	F	-
Sisyrinchium spp.	R	R	-
Solidago spp.	I	I	O
Sorghastrum nutans	FB	FB	RD
Thelypteris palustris	L	L	-
Thuja occidentalis/Juniperus virginiana	R	R	U
Thypa spp.	I	I	S
Triadenum fraseri	Y	Y	R
Triglocan spp.	F	F	R
Unknown spp.	L	L	S

2.6.4   Water level

Water levels varied greatly over the growing season. Ponds 2 and 9, which were historic locations where *E. geniculata* was observed in 2007 and 2014 but where I did not find *E. geniculata* in either 2017 or 2018, exhibited very similar patterns in water level variation (Figure 2.7 and Figure 2.8). In 2017, Pond 2 had an average water depth of 31.3 cm ($\pm$ 0.004, n = 1363) and Pond 9 had an average water depth of 33.0 cm ($\pm$ 0.005, n = 1347). In 2018, Pond 2 had an average water depth of 23.5 cm ($\pm$ 0.008, n = 1429), and Pond 9 had an average water depth of 24 cm ($\pm$ 0.007, n = 1431).

Interestingly, the water levels near Patch 1, where I did observe *E. geniculata* in 2017 and 2018, exhibited a different pattern, exhibiting overall less drawdown but greater day-to-day fluctuations in water depth (Figure 2.8), such that the weekly and monthly amplitude was much greater than at Ponds 2 and 9, particularly at the start of the season (Figure 2.9 and Figure 2.10). Patch 1's pond had an average water depth of 23.6 cm ($\pm$ 0.005, n = 1418) in 2018.

However, average values do not capture the weekly or monthly variability within the growing season. Weekly water level changes at Long Point can be quite drastic. For example, water levels at Patch 1 in 2018 increased from 31.1 cm to 39.8 cm over a seven-day period (Figure 2.9). Water amplitude over the growing season changed as much as 6.8 cm between July and August (Figure 2.10).
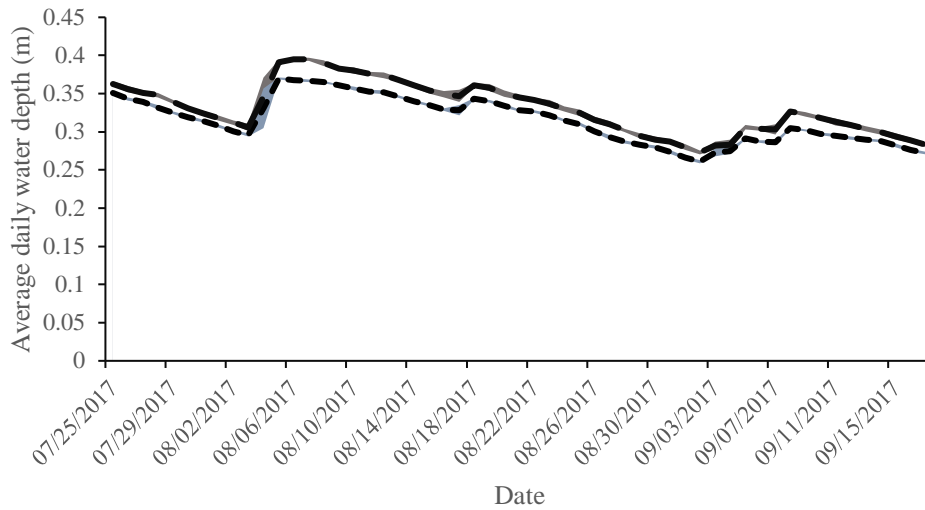
Figure 2.7. Average daily water depth (m) in 2017. Water depth measurements were collected at two historically known locations of *Eleocharis geniculata* (Seasonal average depth at Pond 2 was 0.31 m (n = 24) and the Seasonal average depth at Pond 9 was 0.33 m (n = 24). Shaded error bands show standard deviation.
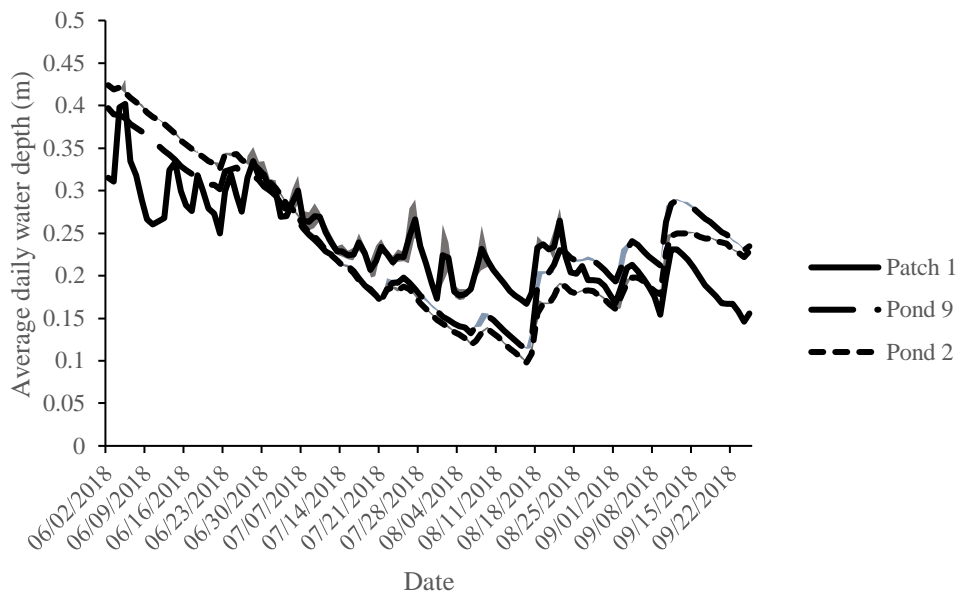

Figure 2.8. Average daily water depth (m) in 2018. Water depth measurements were collected from two historically known locations of *Eleocharis geniculata* (Seasonal average depth at Pond 2 was 0.235 m (n = 12) and Pond 9 was 0.242 m (n = 12) and Patch 1 was 0.236 m (n = 12). Shaded error bands show standard deviation.

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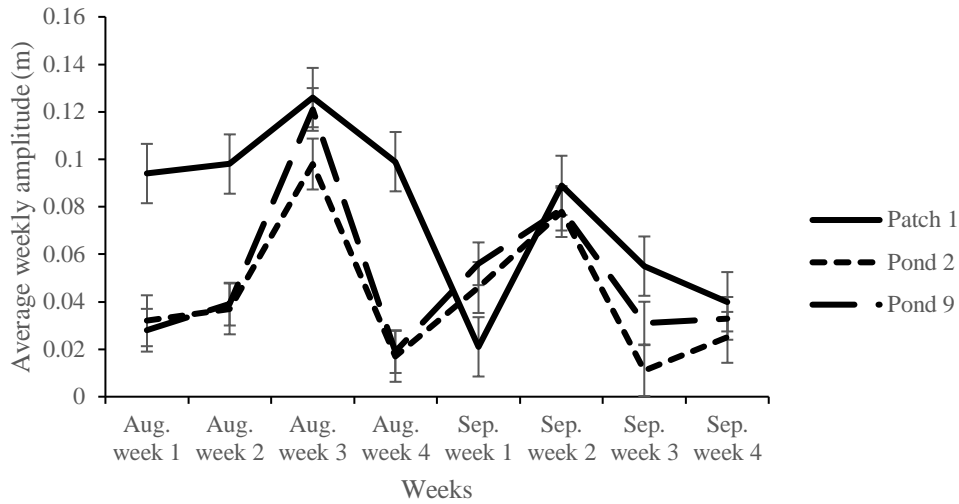
Figure 2.9. Weekly amplitude (m) between August and September in 2018. Water depth measurements were collected at two historically known locations of *Eleocharis geniculata* (Pond 2 (n = 84) and Pond 9 (n = 84)) and the located patch (Patch 1; n = 84) in 2017. Error bars show standard error.
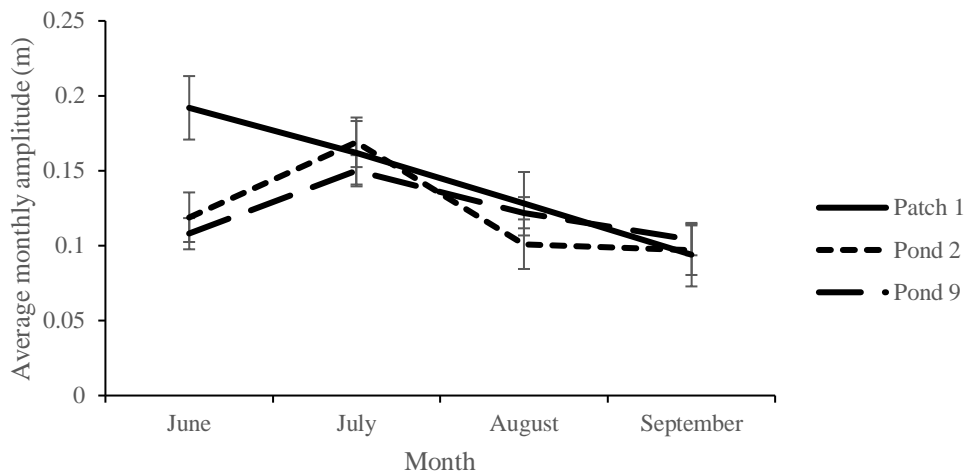
Figure 2.10. Monthly amplitude (m) in 2018 over the growing season. Water depth measurements were collected at two locations where *Eleocharis geniculata* was observed in 2007 and 2014, but not in 2017 or 2018 (Pond 2, June n = 29, July n = 31, August n = 31, and September n = 27) and Pond 9 (n = 1431. June n = 29, July n = 31, August n = 31, and September n = 27)), as well as Patch 1 where it was detected in both 2017 and 2018 (June n = 30, July n = 31, August n = 31 and September n = 25). Error bars show standard error.

2.6.5 Soil quality

2.6.5.1 *Conductivity, temperature, and moisture in 2017*

Two measurements were taken at each of the small sub-patches within Patch 1 of *E. geniculata*, for a total of eight measurements taken in 2017 (Table 2.5). The average soil moisture was 51.24% ($\pm$ 0.82, n = 8). The average temperature was 20.2 °C ($\pm$ 0.23, n = 8). The average conductivity was 45.25 mSm^{-1} ($\pm$ 1.61, n = 8).

Table 2.5. Soil conductivity, temperature and moisture. These measurements were taken at Patch 1 of *Eleocharis geniculata* in 2017. Patch 1 of *E. geniculata* could be split into four small sub-patches separated by other vegetation. Replicate measures were taken to cover the spatial extent of Patch 1, with two measurements taken at each sub-patch.

Eleocharis geniculata sub-patch #	Moisture (%)	Temperature (°C)	Conductivity (mScm^{-1})
1	52.2	21.2	51.0
1	52.4	20.1	46.0
2	52.7	20.3	50.0
2	50.1	19.6	43.0
3	53.0	20.9	47.0
3	50.1	19.3	39.0
4	50.8	20.6	39.0
4	48.6	19.9	47.0

2.6.5.2 *Nutrients, soil moisture and texture 2018*

In 2018, I undertook a more comprehensive characterization of soil quality at the extant *E. geniculata* patches. Soil quality was fairly similar between Patch 1 and Patch 2 (Table 2.6), though Patch 1 had more iron, phosphorus and ammonia than Patch 2. Generally, the soil comprised of moist, bare sand with very low levels of carbon, particularly organic carbon. The pH was slightly alkaline and had relatively high calcium levels. Soil moisture had an average of 31.26 % ($\pm$ 4.02, n = 6). Soil texture consisted of 94.18 % sand ($\pm$ 0.62, n = 6).

Table 2.6. Results from six soil cores spread across the Patches 1 (n = 3) and 2 (n = 3) of *Eleocharis geniculata*, sampled in 2018. For more detailed results, see Appendix (Table S12).

Analyte	units	Average (n = 6)	Standard deviation (n = 6)	Patch 1 (n =3)	Patch 2 (n = 3)
Total Carbon	% dry	2.53	0.37	2.50	2.56
Inorganic Carbon	% dry	1.96	0.21	1.88	2.03
Organic Carbon	% dry	0.58	0.25	0.62	0.54
Extractable NH$_4$	mg/kg dry	4.17	5.89	7.01	1.34
NO$_3$	mg/kg dry	0.45	0.24	0.39	0.50
Soil Moisture	%	31.26	9.85	34.42	28.10
P	mg/L soil dry	2.75	1.12	3.20	2.30
Mg	mg/L soil dry	52.50	22.86	56.00	49.00
K	mg/L soil dry	8.00	5.02	9.00	7.00
Na	mg/L soil dry	24.83	4.75	26.00	23.67
Ca	mg/L soil dry	2750.00	197.48	2766.67	2733.33
Mn	mg/L soil dry	11.52	2.25	10.90	12.13
Zn	mg/L soil dry	0.58	0.44	0.88	0.28
Cu	mg/L soil dry	0.35	0.17	0.43	0.26
Fe	mg/L soil dry	27.83	18.79	38.33	17.33
pH	mg/L soil dry	7.98	0.24	7.90	8.07
Gravel	%	0.20	0.49	0.00	0.40
Total Sand	%	94.18	1.52	93.63	94.73
Sand (very fine)	%	1.88	1.10	2.53	1.23
Sand (fine)	%	55.63	6.29	52.83	58.43
Sand (medium)	%	33.57	6.75	35.70	31.43
Sand (coarse)	%	2.90	1.68	2.46	3.33
Sand (very coarse)	%	0.18	0.31	0.03	0.33
Silt	%	1.60	1.01	1.60	1.60
Clay	%	4.23	1.32	4.76	3.70

2.6.6 Threats from *Phragmites australis*

2.6.6.1 *Mapping*

Both *E. geniculata* Patch 1 and Patch 2 had *P. australis* growing within 100 m of the patch. For Patch 1 in 2017 and in 2018, all *P. australis* growing within the wetland was mapped even outside of the 100 m buffer, as it was growing in potential future habitat of *E. geniculata*. All *P. australis* within the narrow wetland of Patch 2 was also mapped. However, less search effort was allocated to the portion of the 100 m buffer around Patch 2 that lay north of the sand berm, as I determined that any *P. australis* in this area would have difficulty crossing the berm, which was roughly three meters in height, to spread into Patch 2. More, *P. australis* was already growing within this *E. geniculata* patch, suggesting concerns about invasion from the far side of the sand berm was secondary to the existing *P. australis* in the patch. Maps of the distribution of *P. australis* with respect to the location of *E. geniculata* patches are redacted from the online version of this thesis document because the precise location of species at risk is considered sensitive data. However, the maps can be accessed following special request from Dr. Rebecca Rooney at the University of Waterloo (rrooney@uwaterloo.ca).

2.6.7 Water use efficiency and carbon assimilation rate

At photosynthetically active radiation (PAR) rates of > 100 μmol/m^{-2}s^{-1}, *P. australis* has higher water use efficiency than *E. geniculata* (Figure 2.11). Similarly, though at values of incident light < 100 μmol/m^{-2}s^{-1} of PAR, *E. geniculata* loses less carbon than *P. australis.* However, when PAR reaches about 100 μmol/m^{-2}s^{-1}, both species become capable of net carbon assimilation and the rate of carbon assimilation is consistently higher in *P. australis* than in *E. geniculata* (Figure 2.12). Importantly, the amount of PAR reaching the soil beneath a dense *P. australis* stand would prevent *E. geniculata* from net carbon assimilation. If *E. geniculata* received 500 μmol/m^{-2}s^{-1} of sunlight it could grow under sparse *P. australis* (Figure 2.13).
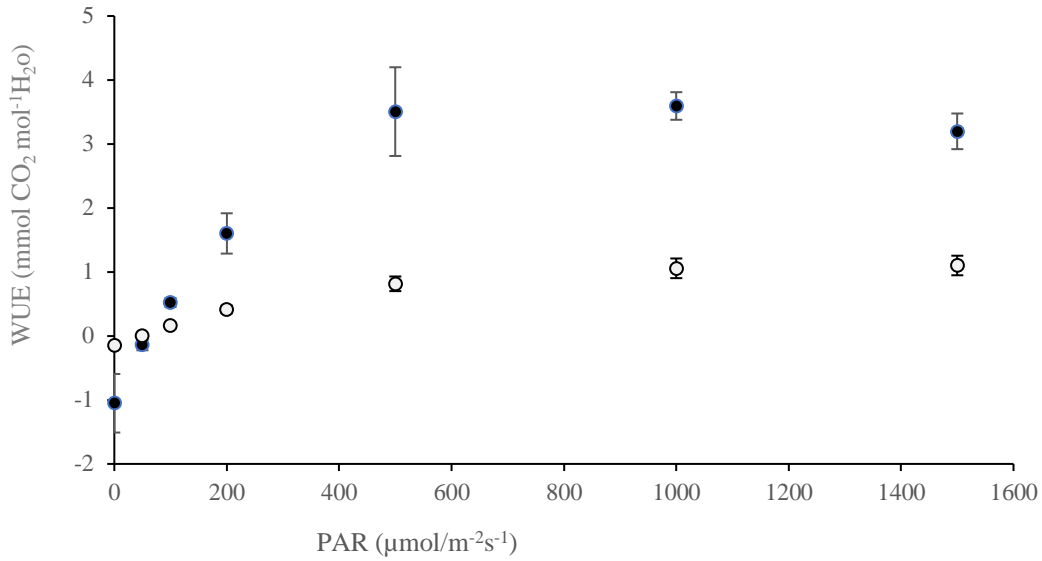
Figure 2.11. Water use efficiency (WUE) and photosynthetically active radiation intensity (PAR) for *Eleocharis geniculata* (hollow circles, n = 10) and *Phragmites australis* (solid circles, n = 10). Error bars show standard error.
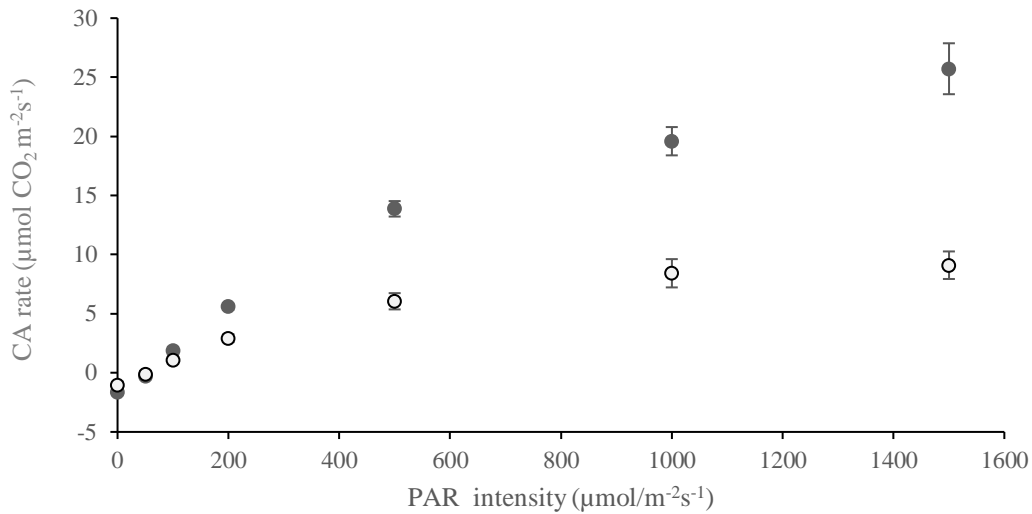

Figure 2.12. Carbon assimilation (CA) rate by *Phragmites australis* (solid circles, n = 10) and *Eleocharis geniculata* (hollow circles, n = 10) in relation to the intensity of photosynthetically active radiation (PAR). Error bars show standard error.

2.6.8 Light availability

The average photosynthetically active radiation (PAR) at the top of the *P. australis* canopy during the mid-day period of measure was 1406.1 µmol/m^{-2}s^{-1} ($\pm$ 143.20, n = 15) and yet only an average 152.3 µmol/m^{-2}s^{-1} ($\pm$ 47.81, n = 15) was reaching the soil, such that the *P. australis* intercepted 89% of incoming PAR (Figure 2.13). These measurements were taken on August 13 and August 14 in 2015. The average PAR measured during mid-day at the top of the *E. geniculata* in Patch 1 was 507.77 µmol/m^{-2}s^{-1} ($\pm$ 69.44, n = 10). The *E. geniculata* measurements were taken on one day (September 27, 2018) and unfortunately in cloudy weather.
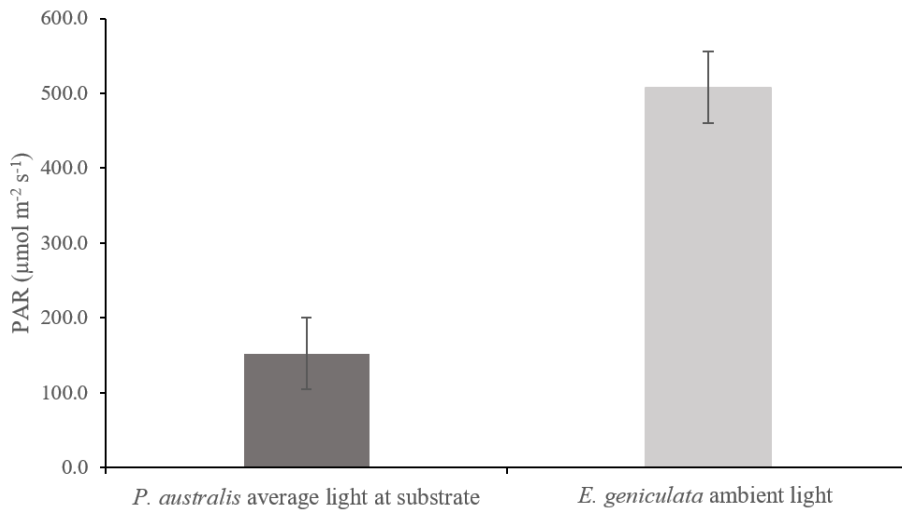

Figure 2.13. Ambient photosynthetic active radiation (PAR) surrounding *Eleocharis geniculata* (n = 10) and PAR that reaches soil in stands of *Phragmites australis* (n = 15). Error bars show standard error.

2.6.9 Stable isotopes

The δ^{15}N value and the δ^{13}C value vary between *E. geniculata* and *P. australis* (Figure 2.14). The δ^{15}N values of *E. geniculata* shoots and roots are lower (between -1.28 ‰ and 1.16 ‰ $\pm$ 0.04, n = 9) than the shoots of *P. australis* (between 1.72 ‰ and 8.63 ‰ $\pm$ 0.08, n = 24). The δ^{13}C values of *E. geniculata* shoots and roots (between - 30.55 ‰ and -27.72 ‰ $\pm$ 3.45, n = 9) are also lower than those of *P. australis* shoots (between -27.11 ‰ and -24.99 ‰ $\pm$ 0.38, n = 24). If we compare only shoots, the average amount of nitrogen (Figure 2.15) in *P. australis* shoots (2.05% by weight $\pm$ 0.08, n = 24) is significantly higher than that of *E. geniculata* shoots (0.98% by weight $\pm$ 0.03, n = 5; t-test: t = 12.378, df = 27, p-value = <0.001). The average amount of

carbon (Figure 2.16) is slightly, but significantly higher in *P. australis* shoots (46.95 % by weight ± 0.38, n = 24) compared to *E. geniculata* shoots (44.00 % by weight ± 0.91, n = 5; t-test: t = 3.199, df = 27, p-value = 0.004).
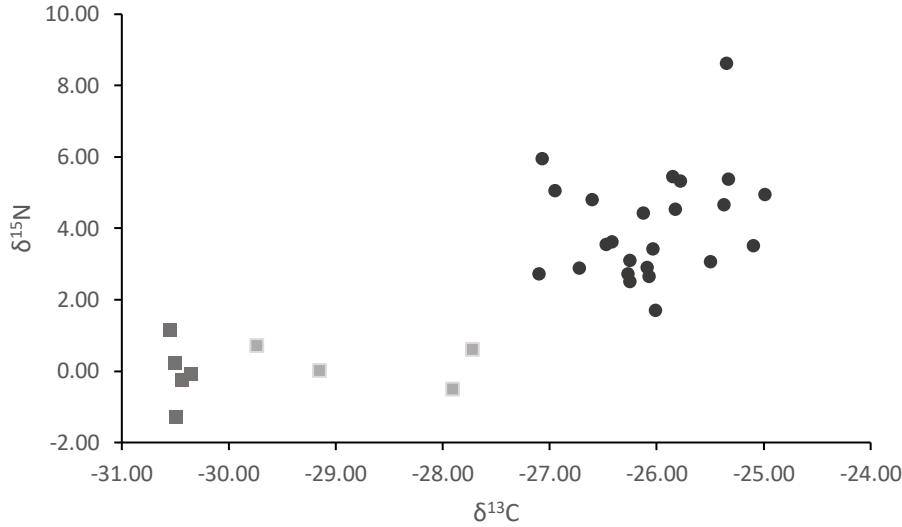

Figure 2.14. Comparison of $\delta^{15}N$ and $\delta^{13}C$ in *Phragmites australis* (black circles, n = 24) and *Eleocharis geniculata* (n = 9, 5 shoots (dark grey squares) and 4 roots (light grey squares).
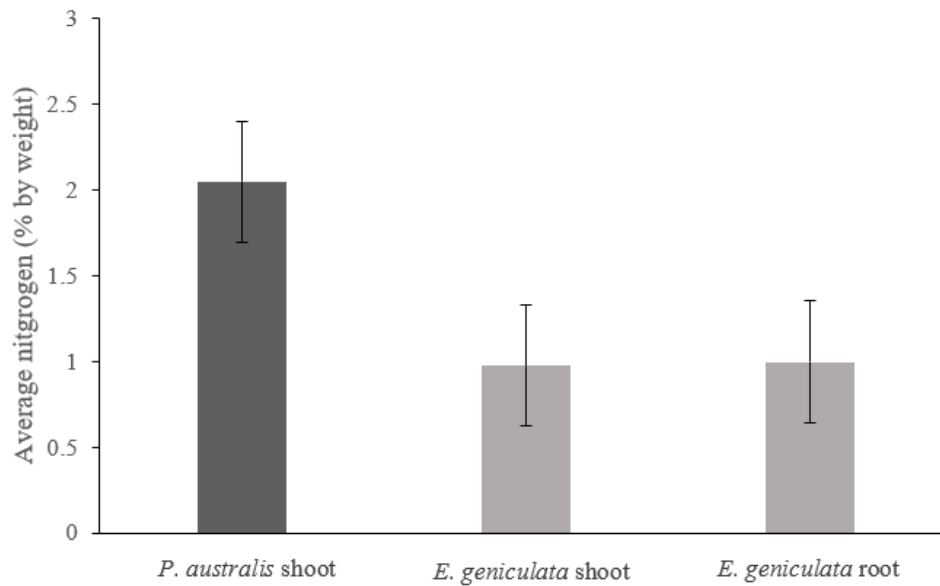

Figure 2.15. Average amount of nitrogen (% by weight) in *Phragmites australis* (n = 24) and *Eleocharis geniculata* (shoots n = 5 and roots n = 4). Error bars show standard error.
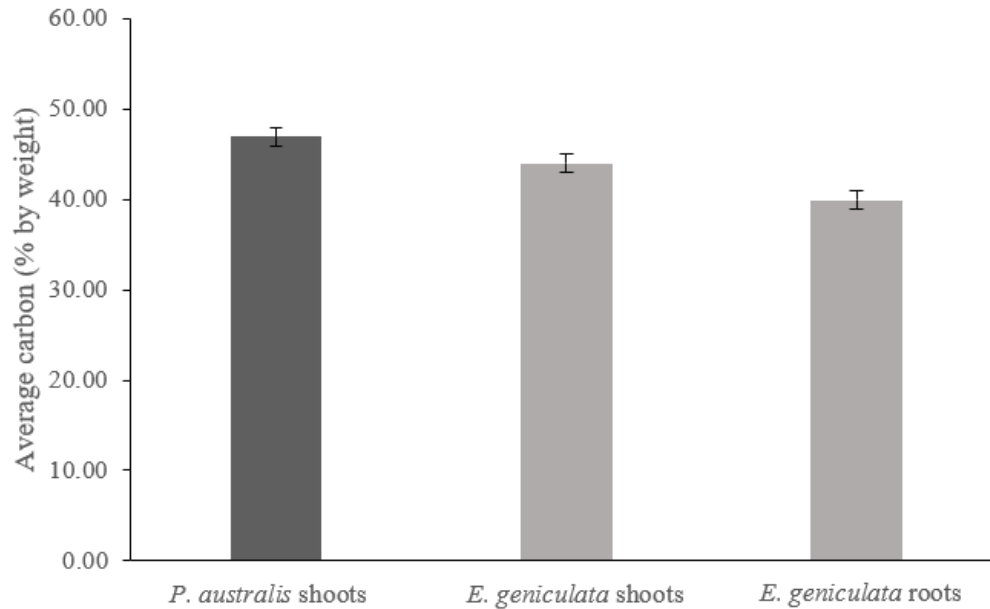
Figure 2.16. Average amount of carbon (% by weight) in *Phragmites australis* (n = 24) and *Eleocharis geniculata* (shoots n = 5 and roots n = 4). Error bars show standard error.

2.7 Discussion

Peripheral populations may be vital to species survival as climate change shifts the distribution of suitable habitat. The Ontario population of *E. geniculata* is a peripheral population of conservation priority (Bowles 2010). However, as noted by the Ontario government recovery strategy, the conservation and recovery of the population is limited by knowledge gaps (Bowles 2010). Of high priority are knowledge gaps pertaining to the current distribution of the population and its size, as well as gaps around its habitat requirements and the threat presented by *P. australis* (Bowles 2010).

My first objective was to locate extant populations and census any I found. Despite surveying 129 wet areas throughout the three locations where *E. geniculata* was reported to occur historically, I located only one patch of *E. geniculata* in 2017 (estimated 44 individuals bearing 712 seed heads; Table 2.2) and two patches in 2018 (combined estimated total 1,407,107 individuals bearing 9,889,735 seed heads and 112,689,953 achenes; Table 2.2). Both of these patches were situated in the Long Point National Wildlife Area on bare sand. Patch 1 was located in a wet area that had historically known points in 2007 from the Ontario Natural Heritage

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Information Center Database (NHIC 2017), but Patch 2 was located in a new location with no historic record of prior occupancy by *E. geniculata*.

Importantly, the patch located in 2017 (Patch 1) is expanding spatially, growing from 6.3 m^2 and 44 individuals to 186 m^2 and 532 individuals in 2018 (Table 2.2). This expansion of *E. geniculata* at Patch 1 may be a result of *P. australis* control actions taken by Birds Canada in this wetland in 2017. Removal of *P. australis* may allow the native plant community to return (Farnsworth and Meyerson 1999) by creating more available habitat and allowing sunlight to reach the seedbank.

I was unable to locate any *E. geniculata* in Rondeau Provincial Park or in Cedar Springs (Table 2.1). Though I am confident based on my survey of the Rondeau Provincial Park that *E. geniculata* has been extirpated from this location, additional surveys in Cedar Springs may be warranted, as I was not able to obtain permission to access all candidate habitat on privately owned land. If possible, a survey of the quarry in Cedar Springs should be undertaken as it is possible the annual *E. geniculata* has colonized the bare sand substrate in this location, but I was denied access permission. More, education and outreach efforts targeting landowners in the Cedar Springs area may garner sightings and increase the willingness of landowners to have their properties surveyed in the future. I created a handout of *E. geniculata* information that can be given to landowners in the Cedar Springs area (Appendix, Figure S1).

My second objective was to characterize *E. geniculata* habitat needs. The sites where I located *E. geniculata* confirmed the habitat requirements outlined in the government recovery strategy (Bowles 2010). In the government recovery strategy, it is mentioned that *E. geniculata* grows in sandy to mud soils (Bowles 2010). However, the *E. geniculata* located during my surveys was only found in sandy soils. The soil analysis found that it was an average of 94.18 % sand ($\pm$ 0.62, n = 6), specifically an average of 55.63 % fine sand ($\pm$ 2.57, n = 6) with very low (<1%) levels of organic carbon and low nutrients (e.g., average P was 2.75 mg/L soil dry $\pm$ 0.46, n = 6; Table 2.6). Secondly, the government recovery strategy explained that *E. geniculata* needs wet soil conditions (Bowles 2010). My research supports this as *E. geniculata* was located in sand with an average of 51.24 % soil moisture ($\pm$ 0.82, n = 8) in 2017 (Table 2.5) and 31.26 % moisture ($\pm$ 4.02, n = 6) in 2018; Table 2.6). The soil was slightly alkaline (average pH 7.98 mg/L soil dry $\pm$0.10, n = 6) and had relatively high average Ca (2750 mg/L soil dry $\pm$ 80.62, n = 6). Finally, the

percent cover of competing vegetation was low, allowing for *E. geniculata* to receive an average of 507.77 μmol/m^{-2}s^{-1} ($\pm$ 69.44, n = 10) of ambient light (Figure 2.13). I attribute this to the high percent of bare sand substrate: considering both patches, the average percent cover of bare sand was 35.3% ($\pm$ 5.24, n = 25), with Patch 1 having an average of 59.8% bare sand ($\pm$ 3.43, n = 10), Patch 2 dense having an average of 24.8% bare sand ($\pm$ 8.26, n = 5), and Patch 2 sparse having an average of 16.0% ($\pm$ 6.49, n = 10).

In both years, I located the patches of *E. geniculata* in late September after many of the co-occurring species had senesced and water levels had dropped (Figure 2.7 and Figure 2.8). This supports the known information from the government recovery strategy that *E. geniculata* is a late growing species (Bowles 2010) and likely requires water drawdown to germinate. This is supported by numerous studies showing that drawdown of water encourages vegetation shifts towards germination of seedbanks (Smith and Kadlec 1983, Keddy and Reznicek 1986, Grewell et al. 2019). For example, at Ponds 2 and 9 (two sites where *E. geniculata* was reported in 2007), I did not detect *E. geniculata* in either 2017 or 2018, likely because water levels were too deep as overall water levels in Lake Erie have been unusually high (National Oceanic and Atmospheric Administration 2020). These two sites had surprisingly high water levels (e.g., Pond 2 in September 2018 had an average water depth of 22 cm ($\pm$ 0.005, n = 27; Figure 2.8) even after late-summer drawdown. With such high water levels, water drawdown in September was not enough to expose bare sand. It is possible that *E. geniculata* would recolonize these locations if lake levels declined to a level more typical of 2007, exposing the bare sand again, presuming that viable propagules remain in the seedbank. The water level at the extant patch (Patch 1), was not only lower than in Ponds 2 and 9, but also more variable, particularly early in the growing season. Patch 1 had much greater weekly and monthly amplitude (for example, Patch 1 had water levels change from 2.1 cm to 8.9 cm between week 1 and week 2 in September compared to Pond 9 with water levels that changed from 5.6 cm to 7.9 cm; Figure 2.9). These fluctuations may be necessary to break seed dormancy. For example, Grabas et al. (2019), found that species richness in meadow marshes was higher with greater daily fluctuations in water levels. This is likely because species have different tolerances to water levels (Keddy and Ellis 1984), so increased fluctuating water levels meets the growing requirements of more species. Highly variable water depths during the early part of the growing season may also prevent dense vegetation growth of co-occurring species, leaving more available bare sand (Keddy and

Reznicek 1986). The germination requirements were met long enough in these fluctuations to encourage germination of *E. geniculata* in Patch 1. More research is needed to establish the importance of early season water-level fluctuations in supporting *E. geniculata* growth.

Species known to co-occur with *E. geniculata* can be used as early-season indictors of where *E. geniculata* could emerge later in the growing season or of habitat capable of supporting *E. geniculata*, even if it is not currently present there. Access to a list of such indicator species would make critical habitat surveys more efficient (Rodrigues and Brooks 2007). This would be extremely valuable for *E. geniculata* conservation, as its late season life history means the window to conduct surveys is narrow and coincides with challenging weather and high winds that limit access to the remote Long Point National Wildlife Area. In 2019, for example, surveys were aborted because high water levels and winds made safe access to survey locations impossible.

There are examples of successful indicator use in the literature. Co-occurring species data can be used in a species distribution model to predict potential habitat (Baumberger et al. 2012, Zossou et al. 2015, Smart et al. 2015) and can be used in part to reintroduce species successfully (Bontrager et al. 2014). For example, Baumberger et al. (2012) predicted suitable habitat for *Linmonium girardianum* (Guss.) by incorporating common co-occurring species into a species distribution model. Indicator species work in these situations because they have similar ecological requirements as the endangered species (Baumberger et al. 2012). Some co-occurring species that could be indicators of potential habitat for *E. geniculata* (Table 2.4) include *Equisetum variegatum* (Schleich.), *Juncus* spp. and *Cyperus esculentus* (L.). These larger, earlier growing, and more easily spotted species could extend the survey period, enabling earlier surveys when weather is more supportive of site access. They also may be used to help predict whether a habitat is likely to be suitable for *E. geniculata* in future restoration programs.

We can also gain information about *E. geniculata*'s habitat needs by looking at locations that it has been excluded from. The majority of surveyed sites in Cedar Springs and Rondeau Provincial Park were highly vegetated, often with *P. australis.* It is therefore perhaps unsurprising that *E. geniculata* appears extirpated from these locations. However, even in the Long Point National Wildlife Area, many candidate and historic habitat locations that I surveyed were invaded by *P. australis*. *Phragmites australis* had invaded 60 of the 129 wet areas I

searched (Table 2.1). It was also growing within 100 m of the extant *E. geniculata* patches I detected in the Long Point National Wildlife Area. In 2017, Patch 1 had over 30 sub-patches of *P. australis* within 100 m, despite on-going control efforts. Once *P. australis* invades, it is known to alter community composition by out competing native plants (Ailstock et al. 2001, Rojas and Zedler 2015), changing the amount of light that hits the seedbank (Meyerson et al. 2000), and disrupting nutrient cycles (Bernal et al. 2017). My research certainly supports the literature identifying *P. australis* as a threat to biodiversity and the conservation of at risk plants like *E. geniculata*. My third objective was to more quantitatively characterize the threat that the invasive lineage of *P. australis* poses to *E. geniculata*. The government recovery strategy notes that *P. australis* is likely the biggest threat facing *E. geniculata* in Ontario (Bowles 2010).

I directly compared the carbon assimilation capacity of *E. geniculata* and *P. australis* across a light intensity gradient. As discussed in the recovery strategy (Bowles 2010) *E. geniculata* tolerates minimal competition for light and my monitoring reveals that it requires a minimum of 100 µmol/m^{-2}s^{-1} of PAR to assimilate carbon above and beyond its respiration needs. A dense canopy of *P. australis* can reach 6 m in height (Meyerson et al. 2000) and my data indicate that it can intercept 89% of incident PAR, such that only an average of 152.3 µmol/m^{-2}s^{-1} ($\pm$ 47.81, n = 15) would reach *E. geniculata* attempting to grow under the *P. australis* canopy, even under sunny skies at mid-day (Figure 2.13). My observations in the sparse sub-patch of Patch 2 reveal that *E. geniculata* is capable of persisting under a low-density canopy of *P. australis*, but I expect that *P. australis* canopy cover greater than 25% would result in its extirpation. Importantly, without intervention to remove *P. australis*, over time its density would likely increase until it shaded out *E. geniculata*. *Eleocharis geniculata*'s peak carbon assimilation is only about 36% that of *P. australis*, even under 1500 µmol/m^{-2}s^{-1} of PAR. This disparity in carbon assimilation levels reveals how asymmetric the competition between the two species is, even if they were equal in height or if *E. geniculata* were somehow exposed to a saturating intensity of PAR. Adding to this conclusion is the observation that subsequent to *P. australis* removal by Birds Canada near Patch 1 in 2017, the extent of Patch 1 increased by a factor of 29 from 6.3 m^2 to 186 m^2. Concomitantly, the population size grew by a factor of 12 from 44 individuals to 532 individuals (Table 2.2). Further substantiating the evidence from the carbon assimilation and light interception data, in locations where I did find *E. geniculata*, it had an

inverse correlation in cover with *P. australis*. As indicated in my NMS ordination, quadrats with higher cover of *P. australis* had lower cover of *E. geniculata* (Figure 2.6).

I also directly compared the water use efficiency of *E. geniculata* and *P. australis* under different light conditions. *Phragmites australis* has higher water use efficiency rates than *E. geniculata* at PAR exposure >100 µmol/m^{-2}s^{-1} (Figure 2.11). Higher water use efficiency shows that *P. australis* is more efficient at creating biomass than *E. geniculata* (Hatfield and Dold 2019). In times of water scarcity, *P. australis* would be better adapted to grow than *E. geniculata* (Hatfield and Dold 2019)*. This is further supported by the differences in the average δ^{13}C value between the two species (Figure 2.14). *Phragmites australis* and *E. geniculata* have a δ^{13}C signature that are within the range of C3 photosynthesis (Kohn 2010), however, *P. australis*' δ^{13}C is higher. Plants with a greater δ^{13}C value discriminate less against ^{13}CO$_2$, implying more efficient photosynthetic processes (Farquhar et al. 1989) using less CO$_2$ per unit of transpired water (Mcalpine et al. 2008).

Further, *P. australis* has a higher N tissue content by weight than *E. geniculata*, (2.05% by weight $\pm$ 0.08, n = 24, compared with 0.98% by weight $\pm$ 0.03, n = 5; Figure 2.15). More, *E. geniculata* has a lower δ^{15}N signature, indicating it is likely nitrogen limited and growing in nitrogen limited soil (Craine et al. 2009). Certainly, the low levels of extracted soil ammonia and nitrate that I observed when I analysed soil from the extant patches of *E. geniculata* would support that interpretation (Table 2.6). In contrast, the *P. australis* tissues I analysed came from the Long Point Provincial Park, where the soil had higher organic content. Because nitrogen is often a limiting resource (Vitousek et al. 1997), *P. australis*' ability to better obtain more nitrogen from its environment increases its competitive ability. Thus, I conclude that a key element of *E. geniculata*'s habitat needs is the absence of competition with *P. australis* and that sustained management of *P. australis* is essential to *E. geniculata*'s recovery.

2.7.1 Future work

For future research I recommend continued monitoring of the extant patches and yearly critical habitat surveys. My results of critical habitat surveys show the fluctuations in the patches from year to year. This makes searching potential habitat yearly important to fully understand population dynamics. Additionally, it would provide information of the stability of the Ontario population. The expansion of Patch 1 between 2017 and 2018 indicates some form of dispersal

of achenes and shows that while adult *E. geniculata* may not be seen every season, achenes could be dormant in the seedbank. However, there remains a knowledge gap in how long the achenes can remain dormant in the seedbank, how many achenes are in the seedbank (Bowles 2010), and their dispersal mechanisms, including the distance that achenes can travel.

Also, a research question for future investigation is how genetically similar or distinct the *E. geniculata* are between Patch 1 and Patch 2. Patch 1 and 2 may be one population with high levels of gene flow, or gene flow between them may be limited. The two patches are spatially distinct, with areas of dry, sand dune habitat and other wetlands located between the two patches. However, *E. geniculata* is likely pollinated by wind, as with many other species in the family Cyperaceae (Goetghebeur 1998), so there could be transfer of pollen between the two patches, depending on the distance of pollen dispersal achieved. Knowing the distance achenes and pollen can travel would assist in evaluating whether the two patches are distinctive or highly related.

Further, a better understanding of the patches' genetic diversity may help explain the differences in morphology, specifically between the dense sub-patch of Patch 2 and the sparse sub-patch in Patch 2 and Patch 1. The dense sub-patches' different growing form could be because of the sparsely invading *P. australis* limiting the amount of sunlight received by *E. geniculata*. The *E. geniculata* dense sub-patches were located on the north side of the wetland, with the sparse *P. australis* growing on the south side of the patches blocking much of the light. Often, species growing in shade environments will put resources into elongated leaves in an attempt to reach light (Van Hinsberg and Van Tienderen 1997). However, late growing species would not receive the benefits of sunlight exposure with longer leaves (Weinig 2000). *Eleocharis geniculata* is a late growing species, so it is possible that the reduced amount of light reaching the seed bank in this area encouraged the shorter growth formations than typically seen in *E. geniculata*. Further, the shorter growth form could be caused by intraspecific competition (Lentz 1999). With so many individuals growing so closely together, competition among *E. geniculata* individuals could account for the shorter height.

Best management practices for controlling *P. australis* in sensitive habitat where plant species at risk may reside need to be developed (Bowles 2010). It is essential that any control of *P. australis* in critical habitat does not damage the seedbank of *E. geniculata*. This is especially important because both *P. australis* and *E. geniculata* are late growing and seed setting species.

If control of *P. australis* occurs before *E. geniculata* sets seed, one entire growing season of achenes could be destroyed if the plants do not reach maturity. It has been shown that management of *P. australis* using herbicides increased native species richness and diversity (Bonello and Judd 2020). My research shows a clear overlap of habitat use between *P. australis* and *E. geniculata*. If *P. australis* spread remains uncontrolled, there may be no available habitat left for *E. geniculata*. However, herbicide-based control must be implemented cautiously to protect any developing *E. geniculata*. To understand the population dynamics of annual plants like *E. geniculata*, that require specific habitat conditions in a highly variable environment, we need long term monitoring. This would answer questions about the fluctuation of the population size year to year. It could also help answer questions about the length of achene dormancy and dispersal mechanisms. Lastly, my study provides anecdotal evidence that *P. australis* removal can increase adult *E. geniculata* patches expand, so identifying best management practices of *P. australis* in *E. geniculata*'s critical habitat could have a huge influence of future *E. geniculata* patches and the continued persistence of the Great Lakes Plains population in Ontario.

3   Conclusion

3.1   Implications and significance

My thesis assists in filling the knowledge gaps in the government recovery strategy and the recovery of *E. geniculata* in Ontario generally. The critical habitat searches show the importance of yearly monitoring to better understand population fluctuations and stability. The habitat measurements provide more specific information on what should be classified as critical habitat for *E. geniculata*. Further, the results can help limit future critical habitat surveys using the more specific criteria, such as searching for co-occurring species. Lastly, my research shows that while *E. geniculata* can survive within sparse *P. australis* stands, *P. australis* is still a major threat to *E. geniculata* in Ontario. *Phragmites australis* was located at over 46% of candidate and historic habitat sites that I surveyed within the designated critical habitat for *E. geniculata*. Further, Patch 1 expansion occurred between 2017 and 2018 while *P. australis* was being controlled in the wetland. These results suggest that control of *P. australis* would likely create more available habitat for *E. geniculata*.

3.2 Future work

To increase available habitat for *E. geniculata* to grow, I recommend testing methods of *P. australis* control that limit the impact on *E. geniculata*. Several methods have been developed to control *P. australis* populations, including flooding, prescribed burns, mechanical cutting, rolling and herbicide application (Norris et al. 2002, OMNR 2011). In Canada, the federal Pest Management Regulatory Authority has approved two herbicides for use in controlling *P. australis*: glyphosate (Visionmax, Monsanto Canada, Winnipeg, MB; Health Canada 2018a) and imazapyr (Arsenal Powerline, BASF, Research Triangle Park, NC; Health Canada 2018b). Both are broad-spectrum, non-selective, post-emergence herbicides so, wide-spread application in *E. geniculata* habitat could put this endangered species at risk. The aerial application of the herbicide glyphosate is recommended in Ontario (OMNR 2011) and has the advantage of being highly effective and well suited to rapid control of large dense stands in remote areas (Mal and Narine 2004).

Best management practices in Ontario dictate that herbicide application should occur in the late fall as the annual above ground tissues of *P. australis* are translocating nutrients down to the perennial rhizomes (OMNR 2011). This period of application has the added benefit of minimizing risk to breeding wildlife and most native plant species which senesce earlier than *P. australis* (OMNR 2011). However, this timing can pose a problem for late senescing species that potentially have not seeded yet (Rodriguez and Jacobo 2013), like *E. geniculata*; particularly if the herbicide used is non-selective, and so spraying near rare plants also puts them at risk (Mal and Narine 2004, Hazelton et al. 2014). For an annual species like *E. geniculata*, the risk of herbicide-induced mortality before the individuals have set seed may threaten the persistence of the entire population. For example, Rodriguez and Jacobo (2013) found that using glyphosate in late summer can lower seedbank diversity and richness, even though glyphosate does not cause seed mortality.

The potential for herbicide-based control of *P. australis* in critical *E. geniculata* habitat to threaten the persistence of any *E. geniculata* makes the aerial application of glyphosate an unacceptable control option. One herbicide-based control method that is more selective than aerial application and less likely to impact non-target species is hand-wicking with either glyphosate or imazapyr herbicides directly onto *P. australis* (Back and Holomuzki 2008). Hand-

wicking occurs by directly rubbing stalks with herbicide using an absorbent glove (OMNR 2011). This form of application is targeted and should cause little harm to surrounding vegetation. The problem with this method is that while it can be effective on small stands of *P. australis* it is labour-intensive and often herbicide application requires multiple visits for multiple years (Ailstock et al. 2001, OMNR 2011). There is also the potential that herbicide translocated into the rhizosphere may affect other nearby plants. This risk has never been investigated, though in the case of *P. australis* control in immediate proximity to *E. geniculata*, such an investigation is warranted. There is also some evidence that imazapyr may accumulate in soils and prevent successful restoration of native flora following invasive species control (Douglass et al. 2016). Although wicking should minimize the accumulation of imazapyr residues in wetland soil, it is unknown if this mechanism could inhibit the recovery of *E. geniculata*.

A second candidate control option that presents minimal risk to native flora and fauna is spading (Short 2017). Short (2017) uses a sharpened spade to cut the *P. australis* stalk from the rhizome at an approximate 45° angle below the soil surface and reports successful reductions in *P. australis* stem density after multiple spadings. This occurs because the rhizome becomes deprived of nutrients and oxygen (due to the removal of aboveground photosynthetic stems) which depletes the resource stores in perennial storage organs and starves the plant (Short 2017). An additional mechanism of stress from spading is that cutting and removing the stems can expose rhizomes to frost, further increasing the stress on the stand (Haslam 1969). Notably, cut stems must be removed and disposed of with care to prevent them from acting as vegetative propagules and further spreading the invasion (Alvarez et al. 2005). Like hand-wicking, this labor-intensive approach to *P. australis* control is not likely feasible for large-scale *P. australis* control. However, it may be effective in small areas where invasion is threatening high conservation value species like *E. geniculata* that would potentially be harmed by alternative control actions. This research would have far reaching applications because 31 other species at risk in Ontario list *P. australis* control to aid in recovery (Bickerton 2015).

Future potential research for *E. geniculata* could test how susceptible *E. geniculata* is to glyphosate, which is the main herbicide used in controlling *P. australis*. It could also identify the main dispersal methods of achenes and the distance that cross-pollination can occur to inform

how genetically connected patches of *E. geniculata* are and what candidate habitat it could reach. It would also be valuable to conduct vegetation surveys at historic points of occurrence where *E. geniculata* is no longer located to create a joint species distribution model. *Eleocharis geniculata* may be able to persist in the seed bank despite herbicide application. If that is the case, the two more labour-intensive options may not be necessary as long as herbicide application happens after *E. geniculata* has set achenes. As mentioned in chapter 2, we do not know if the two patches are the same population. Knowing the distance that pollination can occur would identify if the patches are the same or separate populations and could provide more information on habitat requirements if the two patches were studied more in depth. Lastly, a joint species distribution models involves combining data on environmental variables and biological variables to predict species distributions (Pollock et al. 2014). This would be useful because the model can differentiate if the relationship among the co-occurring species is related to environmental variables or interactions among the species (Pollock et al. 2014). A joint species distribution model for *E. geniculata* would make predicting potential habitat easier and would reduce the amount of area necessary to complete comprehensive searches.

Continued monitoring of the *E. geniculata* patches in Ontario and increasing habitat availability through control of *P. australis* is needed for the recovery of *E. geniculata*. Conserving the Great Lakes Plains population of *E. geniculata* is a priority. This is because *E. geniculata* adds to the biodiversity of wetlands and the mosaic patchwork of ecosystems as a whole. Any loss of biodiversity is concerning because as the climate changes, only certain species will be able to adjust and thrive, and those species are currently unknown (Hoffmann and Sgró 2011). Loss in biodiversity limits the resilience of ecosystems (Chapin et al. 2000) and resilient ecosystems are more likely to recover and withstand climate change (Isbell et al. 2015). Lastly, rare species can provide an ecosystem function that is disproportional to their population size (Mouillot et al. 2013) making them a conservation priority.

4 <u>References</u>

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5 **Appendix**

Table S1. Cedar Springs critical habitat survey results. N/C represents measurements that were not collected. SA* represents sand exposed with limited vegetation and more likely to provide suitable habitat for *Eleocharis geniculata*.

Pond (P)/ wet area (WA)	Search Date	*Phragmites australis* present? (Y/N)	*Eleocharis* spp. Present? (Y/N)	Sand (SA; SA* if bare sand)/ Organic soil (OS)	Water depth (cm)
P 3	14-Jun-17	Y	Y	SA*	24
P 11	15-Jun-17	N	Y	SA*	N/C
P 13	15-Jun-17	Y	Y	SA*	N/C
P 15	15-Jun-17	Y	Y	SA*	N/C
P 16	16-Jun-17	N	Y	SA	N/C
P 17	14-Jun-17	Y	Y	SA	0
P 18	18-Jul-17	N	Y	SA	N/C
P 19	16-Jun-17	Y	Y	SA	N/C
P 20	15-Jun-17	Y	Y	SA*	N/C
P 21	16-Jun-17	Y	Y	SA	N/C
P 22	16-Jun-17	Y	Y	SA	N/C
P 23	15-Jun-17	N	Y	SA*	N/C
P 24	15-Jun-17	N	Y	SA*	N/C
P 26	14-Jun-17	Y	Y	SA	N/C
P 27	18-Jul-17	N	N	SA	N/C

Pond (P)/ wet area (WA)	Search Date	*Phragmites australis* present? (Y/N)	*Eleocharis* spp. Present? (Y/N)	Sand (SA; SA* if bare sand)/ Organic soil (OS)	Water depth (cm)
P 28	14-Jun-17	N	Y	SA	N/C
P 29	16-Jun-17	N	Y	SA	N/C
P 3	14-Jun-17	Y	Y	SA*	24
WA 1	14-Jun-17	Y	Y	SA*	7
WA 2	18-Jul-17	N	N	SA	N/C
WA 3	15-Jun-17	Y	N	SA	0
WA 4	15-Jun-17	Y	N	SA	0
WA 5	15-Jun-17	N	N/C	SA	0
WA 6	15-Jun-17	N	Y	SA	N/C
WA 8	18-Jul-17	N	N	SA	0

Table S2. Rondeau Provincial Park critical habitat survey. SA* represents sand exposed with limited vegetation and more likely to provide suitable habitat for *Eleocharis geniculata*.

Pond (P)/ wet area (WA)	Date	*Phragmites australis* present? (Y/N)	*Eleocharis* spp. Present? (Y/N)	Sand (SA; SA* if bare sand)/ Organic soil (OS)	Water depth (cm)
WA 1	10-Sep-2018	N	N	OS	43
WA 2	10-Sep-2018	Y	Y	OS	9
WA 3	10-Sep-2018	N	N	OS	27
WA 4	10-Sep-2018	N	N	OS	10
WA 5	10-Sep-2018	N	N	OS	12
WA 6	10-Sep-2018	N	N	OS	10
WA 7	10-Sep-2018	Y	N	OS	10.5
WA 8	10-Sep-2018	N	N	OS	25
WA 9	10-Sep-2018	N	N	OS	6
WA 10	10-Sep-2018	Y	N	OS	4
WA 11	10-Sep-2018	N	N	OS	8
WA 12	10-Sep-2018	N	N	OS	10
WA 13	10-Sep-2018	Y	N	SA	13
WA 14	10-Sep-2018	Y	N	SA*	1
WA 15	10-Sep-2018	N	N	SA*	10
WA 16	10-Sep-2018	Y	N	SA*	20

Pond (P)/ wet area (WA)	Date	*Phragmites australis* present? (Y/N)	*Eleocharis* spp. Present? (Y/N)	Sand (SA; SA* if bare sand)/ Organic soil (OS)	Water depth (cm)
WA 17	10-Sep-2018	N	N	SA*	2-10 (variable)
WA 18	10-Sep-2018	N	N	SA	24
WA 20	10-Sep-2018	N	N	SA	13
WA 21	10-Sep-2018	N	N	SA	11
WA 22	10-Sep-2018	N	N	SA	31

Table S3. Long Point National Wildlife Area critical habitat search 2017. N/C represents measurements that were not taken. SA* represents sand exposed with limited vegetation and more likely to provide suitable habitat for *Eleocharis geniculata*.

Pond (P)/ wet area (WA)	Date	*Phragmites australis* present? (Y/N)	*Eleocharis* spp. Present? (Y/N)	Sand (SA; SA* if bare sand)/ Organic soil (OS)	Water depth (cm)
P 2	20-Jun-17	Y	Y	SA	26.5
P 10	22-Jun-17	Y	N	OS	NC
P 11	22-Jun-17	Y	Y	SA	NC
P 13	23-Jun-17	Y	Y	SA	NC
P 14	23-Jun-17	Y	N	SA	NC
P 3	22-Jun-17	N	Y	SA	NC
P 4	22-Jun-17	Y	Y	SA	NC
P 7	22-Jun-17	Y	Y	SA*	20
P 8	22-Jun-17	N	Y	SA	NC
P 9	22-Jun-17	Y	Y	SA	NC
WA 10	22-Jun-17	N	Y	SA	NC
WA 11	22-Jun-17	N	Y	SA	NC
WA 14	22-Jun-17	N	Y	SA	NC
WA 15	22-Jun-17	N	N	SA*	0
WA 16	22-Jun-17	N	N	SA*	0
WA 2	22-Jun-17	Y	N	SA	NC

Pond (P)/ wet area (WA)	Date	*Phragmites australis* present? (Y/N)	*Eleocharis* spp. Present? (Y/N)	Sand (SA; SA* if bare sand)/ Organic soil (OS)	Water depth (cm)
WA 21	22-Jun-17	Y	Y	SA	NC
WA 22	22-Jun-17	N	Y	SA	NC
WA 23	22-Jun-17	N	N	SA*	0
WA 25	22-Jun-17	Y	Y	SA	NC
WA 26	23-Jun-17	Y	Y	SA*	NC
WA 28	22-Jun-17	N	Y	SA	NC
WA 29	22-Jun-17	N	N	SA	21
WA 3	22-Jun-17	Y	N	OS	NC
WA 30	22-Jun-17	N	Y	SA	NC
WA 32	22-Jun-17	N	N	SA	NC
WA 40	22-Jun-17	N	N	SA	0
WA 43	23-Jun-17	Y	N	SA	NC
WA 45	23-Jun-17	N	Y	SA	NC
WA 46	23-Jun-17	N	Y	SA	NC
WA 47	23-Jun-17	Y	N	SA	15
WA 60	22-Jun-17	N	N	SA	NC
WA 62	23-Jun-17	Y	Y	SA	40
WA 63	23-Jun-17	Y	Y	SA*	NC
WA 64	23-Jun-17	Y	Y	SA*	NC

Pond (P)/ wet area (WA)	Date	*Phragmites australis* present? (Y/N)	*Eleocharis* spp. Present? (Y/N)	Sand (SA; SA* if bare sand)/ Organic soil (OS)	Water depth (cm)
WA 66	23-Jun-17	Y	Y	SA	NC
WA 67	23-Jun-17	N	Y	SA*	NC
WA 68	23-Jun-17	Y	Y	SA	NC
WA 69	23-Jun-17	Y	Y	SA	NC
WA 71	23-Jun-17	Y	N	SA*	NC
WA 73	23-Jun-17	N	Y	SA	NC

Table S4. Long Point National Wildlife Area critical habitat survey in 2018. SA* represents sand exposed with limited vegetation and more likely to provide suitable habitat for *Eleocharis geniculata*.

Pond (P)/ wet area (WA)	Date	*Phragmites australis* present? (Y/N)	*Eleocharis* spp. Present? (Y/N)	Sand (SA; SA* if bare sand)/ Organic soil (OS)	Water depth (cm)
P 01	28-Sep-18	Y	N	SA	29
P 02	28-Sep-18	Y	Y	SA	32.5
P 03	28-Sep-18	Y	N	SA	18
P 04	28-Sep-18	Y	Y	SA	29.5
P 05	28-Sep-18	Y	N	SA	45
P 06	28-Sep-18	Y	N	SA	32.5
P 07	28-Sep-18	Y	N	SA*	45.5
P 08	28-Sep-18	Y	N	SA	20
P 09	28-Sep-18	Y	Y	SA	20.5
P 10	28-Sep-18	Y	Y	OS	49
P 11	29-Sep-18	Y	Y	SA	40
P 12	29-Sep-18	Y	Y	SA	63
P 13	29-Sep-18	Y	N	SA	31
P 14	30-Sep-18	Y	Y	SA	57.5
WA 01	28-Sep-18	N	N	SA	20
WA 02	28-Sep-18	Y	N	SA	17

Pond (P)/ wet area (WA)	Date	*Phragmites australis* present? (Y/N)	*Eleocharis* spp. Present? (Y/N)	Sand (SA; SA* if bare sand)/ Organic soil (OS)	Water depth (cm)
WA 03	28-Sep-18	N	N	OS	20
WA 04	28-Sep-18	Y	N	SA	16.5
WA 05	28-Sep-18	N	Y	SA	0
WA 06	28-Sep-18	N	Y	SA	0
WA 07	28-Sep-18	N	N	SA	0
WA 08	28-Sep-18	N	N	SA	5.5
WA 09	28-Sep-18	N	Y	SA	5
WA 10	28-Sep-18	N	N	SA	7
WA 11	28-Sep-18	N	Y	SA	2
WA 12	28-Sep-18	Y	N	SA*	25
WA 13	28-Sep-18	N	N	SA	10.5
WA 14	30-Sep-18	N	Y	SA	0
WA 15	28-Sep-18	N	N	SA*	0
WA 16	28-Sep-18	N	N	SA*	0
WA 17	28-Sep-18	Y	N	SA	19.5
WA 18	28-Sep-18	N	N	SA	24
WA 19	28-Sep-18	N	Y	SA	4.5
WA 20	28-Sep-18	N	N	SA	16.5
WA 21	28-Sep-18	N	Y	SA	1.5

Pond (P)/ wet area (WA)	Date	*Phragmites australis* present? (Y/N)	*Eleocharis* spp. Present? (Y/N)	Sand (SA; SA* if bare sand)/ Organic soil (OS)	Water depth (cm)
WA 23	28-Sep-18	N	N	SA*	0
WA 24	29-Sep-18	Y	N	SA	32.5
WA 25	29-Sep-18	Y	Y	SA	20.5
WA 26	29-Sep-18	Y	N	SA*	0
WA 27	29-Sep-18	N	Y	SA	3
WA 28	29-Sep-18	N	Y	SA	16.5
WA 29	29-Sep-18	N	Y	SA	17
WA 30	29-Sep-18	N	Y	SA	3
WA 33	29-Sep-18	Y	Y	SA	3
WA 34	29-Sep-18	N	Y	SA	4.5
WA 35	29-Sep-18	Y	Y	SA*	15
WA 36	29-Sep-18	Y	N	SA	10
WA 37	29-Sep-18	N	Y	SA*	19.5
WA 40	29-Sep-18	N	Y	SA	0
WA 41	29-Sep-18	N	Y	SA	0
WA 42	29-Sep-18	Y	N	SA	13
WA 43	29-Sep-18	Y	N	SA	35.5
WA 44	30-Sep-18	Y	N	SA*	0
WA 45	30-Sep-18	N	Y	SA	9

Pond (P)/ wet area (WA)	Date	*Phragmites australis* present? (Y/N)	*Eleocharis* spp. Present? (Y/N)	Sand (SA; SA* if bare sand)/ Organic soil (OS)	Water depth (cm)
WA 46	30-Sep-18	N	Y	SA	8
WA 47	30-Sep-18	N	Y	SA	0.5
WA 48	29-Sep-18	N	Y	SA	9.5
WA 49	29-Sep-18	N	N	SA	4
WA 50	29-Sep-18	N	Y	SA	11
WA 51	29-Sep-18	N	Y	SA	11.5
WA 31/32	29-Sep-18	N	Y	SA	1.5
WA 52	29-Sep-18	Y	N	SA	11.5
WA 53	29-Sep-18	Y	Y	SA	15
WA 54	30-Sep-18	N	Y	SA*	0
WA 55	30-Sep-18	Y	Y	SA	3.5
WA 56	30-Sep-18	N	Y	SA	6.5
WA 57	30-Sep-18	Y	Y	SA	10
WA 58	30-Sep-18	Y	Y	SA	3.5
WA 59	30-Sep-18	Y	Y	SA*	0
WA 60	29-Sep-18	N	Y	SA	7.5
WA 61	29-Sep-18	N	Y	SA	0
WA 62	26-Sep-18	Y	Y	SA	33.5
WA 63	26-Sep-18	Y	Y	SA*	33.5

Pond (P)/ wet area (WA)	Date	*Phragmites australis* present? (Y/N)	*Eleocharis* spp. Present? (Y/N)	Sand (SA; SA* if bare sand)/ Organic soil (OS)	Water depth (cm)
WA 64	26-Sep-18	Y	N	SA*	21
WA 65	26-Sep-18	N	N	SA	0
WA 66	01-Oct-18	Y	N	SA	34.5
WA 67	01-Oct-18	Y	Y	SA*	15
WA 68	01-Oct-18	Y	N	SA	18
WA 69	01-Oct-18	N	Y	SA	7.5
WA 71	01-Oct-18	Y	N	SA*	28
WA 72	01-Oct-18	Y	N	SA	19

Table S5. Long Point National Wildlife Area survey of sites with historical *Eleocharis geniculata* occurrence records. Sites reflect point locations where historical records exist for *E. geniculata* populations. Comprehensive searches of the entire surrounding ponds or wet areas were not undertaken on this visit due to time constraints. Instead, a selective search of potential habitat within a 50 m buffer around each point was searched in detail. N/C represents information that was not collected.

Site ID	Date	Water Depth (cm)	Presence of *Phragmites australis*? (Y/N)	*Eleocharis* spp. Present? (Y/N)	Canopy Height Estimate (cm)	Notes *Phrag. Means *Phragmites australis*
2014 BS	19-Sep-17	36	Y	Y	72	Small amount of Phrag. on East end of pond, not near point
LPS 1	19-Sep-17	30	N	Y	95	Algae covering most bare spots
LPS 2	19-Sep-17	41	N	Y	N/C	Algae covering most bare spots
LPS 3	19-Sep-17	0	N	N	0	Dry, on path
LPS 4	19-Sep-17	18	Y	Y	N/C	No bare sand
LPS 5	18-Sep-17	21	Y	Y	88	Minimal bare sand, heavily vegetated to the waterline
LPS 6	18-Sep-17	20	Y	Y	300	Very dense Phrag.
LPS 7	18-Sep-17	53	Y	N	300	Very dense Phrag.

Site ID	Date	Water Depth (cm)	Presence of *Phragmites australis*? (Y/N)	*Eleocharis* spp. Present? (Y/N)	Canopy Height Estimate (cm)	Notes *Phrag. Means *Phragmites australis*
LPS 8	18-Sep-17	51	Y	Y	N/C	Dense Phrag., minimal bare sand
LPS 9	18-Sep-17	15	Y	Y	66	Bare sand on islands
LPS 10	18-Sep-17	46	Y	Y	N/C	Bare sand
LPS 11	18-Sep-17	25	Y	Y	N/C	Minimal bare sand, heavily vegetated to the waterline
LPS12	18-Sep-17	0	Y	N	N/C	Dry
LPS 13	18-Sep-17	21	Y	Y	88	Minimal bare sand, heavily vegetated to the waterline
LPS 14	18-Sep-17	N/C	Y	N	N/C	Was not checked but very close to LPS 12
LPS 15	18-Sep-17	N/C	N	Y	N/C	Was not checked but very close to LPS 27
LPS 16	18-Sep-17	26	Y	N	400	Dense Phrag.
LPS 17	19-Sep-17	28	N	Y	85	Sparse canopy, bare spots underwater
LPS 18	18-Sep-17	11	Y	Y	340	Phrag. then dry meadow
LPS 19	18-Sep-17	46	Y	Y	N/C	Bare sand

Site ID	Date	Water Depth (cm)	Presence of *Phragmites australis*? (Y/N)	*Eleocharis* spp. Present? (Y/N)	Canopy Height Estimate (cm)	Notes *Phrag. Means *Phragmites australis*
LPS 20	18-Sep-17	20	Y	Y	300	Very dense Phrag.
LPS 21	19-Sep-17	40	N	Y	89	Potential if water levels drop
LPS 22	19-Sep-17	45	Y	N	N/C	Organic soil, dense Phrag.
LPS 23	19-Sep-17	41	N	Y	N/C	Algae covering most bare spots
LPS 24	19-Sep-17	11	N	Y	0	Lots of bare sand
LPS 25	19-Sep-17	0	N	N	N/C	Dry, surrounded by organic forest
LPS 26	18-Sep-17	53	Y	N	300	Very dense Phrag.
LPS 27	19-Sep-17	22	N	Y	0	Algae covering most bare spots
LPS 28	19-Sep-17	22	N	Y	65	Lots of bare sand

Species at risk Fact Sheet: Bent Spike-rush (*Eleocharis geniculata*)

Bent Spike-rush

Habitat: Sandy, wet shores of ponds and lakes, with minimal vegetation canopy.

Description: A small (less than 20 cm) sedge, growing in a clump formation, with black seeds in late summer.

Importance: Ontario has the northern most population of Bent Spike-rush in North America. As climate change continues, these populations could be vital to the species survival.

Main threat: Habitat loss and invasive European Common Reed (*Phragmites australis*).

European Common Reed

Habitat: wet areas, all soil types

Description: Invasive perennial grass, growing up to 6 m tall.

Impact: This grass has the ability to expand rapidly into newly disturbed areas and outcompete native vegetation, decreasing biodiversity of wetlands and wildlife.

Control: If European Common Reed is on your property, consider visiting Ontario Phragmites Working Group website (www.opwg.ca) for control information.

Found Bent Spike-rush?

If you think you found Bent Spike-rush please report to the Natural Heritage Information center (www.ontario.ca/page/natural-heritage-information-centre)

Figure S1. Cedar Springs *Eleocharis geniculata* handout.

Table S6. Percent cover estimates of vegetation and other cover types co-occurring with *Eleocharis geniculata* in Patch 1 in 2018. The surveys were completed using ten 0.25 m^2 quadrats.

	Quadrats									
Species	1	2	3	4	5	6	7	8	9	10
	Percent cover									
Eleocharis spp.	5	-	5	-	-	-	-	-	-	-
Eleocharis geniculata	5	2.5	5	1	7.5	15	7.5	2.5	15	15
Typha spp.	5	2.5	-	-	2.5	-	-	-	-	-
Potamageton spp.	2.5	-	-	-	-	-	-	-	-	-
Juncus spp.	33	10	2.5	-	15	-	-	5	5	1
Bare sand	50	66	66	75	50	50	66	50	75	50
Juncus marginatus	-	2.5	15	5	-	-	-	-	-	-
Equisetum variegatum	-	15	10	-	15	0.01	-	0.01	1	1
Hypericum kalmianum	-	0.25	1	-	-	-	-	-	-	-
Eleocharis flavescens	-	2.5	2.5	0.25	-	-	-	1	-	-
Carex spp.	-	-	1	-	-	-	-	-	-	5
Solidago spp.	-	-	2.5	-	-	-	-	-	-	-
Schoenoplectus pungens	-	-	-	0.25	-	-	-	-	-	-
Alisma spp.	-	-	-	5	2.5	7.5	15	15	-	2.5
Cyperus esculentus	-	-	-	15	5	10	7.5	5	2.5	-
Chara spp.	-	-	-	1	-	-	-	5	1	-

Phragmites australis	-	-	-	-	2.5	-	-	1	-	-
Sagittaria spp.	-	-	-	-	-	7.5	2.5	2.5	5	5
Helenium autumnale	-	-	-	-	-	5	-	-	-	-
Unknown spp.	-	-	-	-	-	0.25	-	-	-	0.25
Triglochin spp.	-	-	-	-	-	-	-	2.5	-	-
Bryophyta spp.	-	-	-	-	-	-	-	5	-	-
P. australis litter	-	-	-	-	-	-	-	-	-	5

Table S7. Percent cover estimates of vegetation and other cover types co-occurring with *Eleocharis geniculata* in Patch 2 dense sub-patches in 2018. The surveys were completed using five 0.25 m^2 quadrats.

Species	Quadrats				
	11	12	13	14	15
	Percent cover				
Eleocharis geniculata	50	33	50	66	33
Bare sand	15	1	33	25	50
Equisetum variegatum	5	1	-	-	-
Carex spp.	-	-	1	-	5
Cyperus esculentus	-	-	-	-	7.5
Chara spp.	-	1	-	-	-
Aster spp.	5	-	10	2.5	5
Juncus brevicaudatus	2.5	2.5	1	1	2.5
Water	15	66	-	10	-
Panicum spp.	-	1	-	2.5	-
Juncus balticus/ Juncus arcticus	-	-	1	-	-
Parnassia palustris	-	-	-	-	2.5

Table S8. Percent cover estimates of vegetation and other cover types co-occurring with *Eleocharis geniculata* in Patch 2 sparse sub-patches in 2018. The surveys were completed using ten 0.25 m² quadrats.

	Quadrats									
Species	16	17	18	19	20	21	22	23	24	25
	Percent cover									
Eleocharis geniculata	2.5	1	2.5	1	2.5	5	0.25	2.5	25	1
Typha Spp.	-	-	-	-	-	-	-	-	-	0.25
Juncus spp.	0.25	-	-	-	0.25	-	1	0.25	-	15
Bare sand	50	10	-	-	25	-	-	25	-	50
Equisetum variegatum	33	25	1	66	25	5	-	33	15	7.5
Carex spp.	-	15	-	-	5	-	0.25	-	-	15
Cyperus esculentus	10	0.25	-	-	-	-	-	-	-	-
Chara spp.	-	-	-	-	-	-	-	-	1	-
Phragmites australis	5	10	15	-	15	5	15	7.5	-	5
Unknown spp.	-	-	0.25	0.25	-	-	-	-	-	-
Aster spp.	2.5	1	-	-	-	2.5	2.5	-	0.25	1
Juncus brevicaudatus	-	5	2.5	-	-	-	-	-	-	0.25
Water	-	-	66	33	25	66	-	25	66	5
Panicum spp.	-	1	-	-	-	-	-	-	-	-
Juncus balticus/ Juncus arcticus	1	-	10	1	-	15	-	-	-	-
Litter	-	5	-	-	2.5	1	-	2.5	-	2.5

Species	Quadrats									
	16	17	18	19	20	21	22	23	24	25
	Percent cover									
Parnassia palustris	-	5	-	-	-	-	-	-	-	0.25
Standing dead	-	25	1	-	-	10	-	-	-	1
Utricularia vulgaris	-	-	5	0.25	-	-	-	-	-	-
Populus deltoides	-	-	-	-	-	-	1	-	-	-
Eleocharis palustris	-	-	-	-	-	-	7.5	-	-	1

Table S9. June 28, 2018 phenological surveys of co-occurring species with *Eleocharis geniculata*. The LPS (Long Point Spike-rush) points (collected in 2007) and *E. geniculata* 2014 (located in Pond 2) are historically known populations of *E. geniculata*. I only used LPS points locate in Pond 2 and Pond 9 for these surveys. Patch 1 is *E. geniculata* Patch 1 in 2018. Y= young leaves, I= increasing leaf size, L=leaves, FB= flower/flower bud, O= open flower, F= fruit, R= ripe fruits/seeds.

Species	*E. geniculata* 2014	Patch 1	LPS 9	LPS 10	LPS 12	LPS 15	LPS 17	LPS 24
Liatris cylindracea	-	FB	-	-	-	-	-	-
Solidago spp.	-	I	-	-	-	-	-	-
Poa spp.	-	FB	-	-	-	-	-	-
Panicum flexile	-	FB	-	-	-	-	-	-
Juniperus horizontalis	L	-	-	-	-	L	L	-
Schenoplectus spp.	-	-	-	-	-	-	-	FB
Sorghastrum nutans	FB	-	L	-	L	FB	FB	FB
Juncus spp.	FB	FB	-	-	O	FB	FB	-
Triglocan spp.	F	-	F	FB	FB	-	Y	F
Eupatorium perfoliatum	L	-	L	-	-	-	-	-
Phragmites australis	-	L	FB	-	FB	-	-	-
Artemisia biennis	-	L	-	-	-	-	-	-
Equisetum spp.	-	FB	-	I	-	-	-	-

Species	*E. geniculata* 2014	Patch 1	LPS 9	LPS 10	LPS 12	LPS 15	LPS 17	LPS 24
Carex aurea	-	R	-	-	-	-	-	-
Carex buxbaumii	-	R	FB	-	F	F	F	-
Carex pellita/Carex lasiocarpa	-	F	-	-	-	-	-	-
Unknown spp.	L	L	L	L	L	L	L	L
Melilotus albus	-	FB	-	-	-	-	-	-
Lathryus palustris	-	O	-	-	-	-	-	-
Thuja occidentalis/Juniperus virginiana	-	F	-	L	RF	-	-	-
Fragaria virginiana	-	L	-	-	L	-	-	-
Polygonum/ Potamageton spp.	-	L	-	L	-	-	-	-
Hypericum perforatum	I	I	Y	-	-	-	-	-
Castilleja coccinea	-	FB	-	-	FB	-	-	-
Sisyrinchium spp.	-	R	-	-	-	-	-	-
Medicago lupulina	-	O	-	-	-	-	-	-
Schoenoplectus pungens	-	-	O	L	-	-	-	-
Scirpus microcarpus	-	F	-	-	-	-	-	-

Species	E. geniculata 2014	Patch 1	LPS 9	LPS 10	LPS 12	LPS 15	LPS 17	LPS 24
Rosa palustris	L	-	-	-	-	L	-	-
Triadenum fraseri	-	-	-	-	-	Y	-	-
Lysimachia thyrsiflora	-	-	-	L	L	-	-	L
Thelypteris palustris	L	-	L	-	-	L	L	-
Thypa spp.	-	-	-	I	-	-	-	-
Pontederia cordata	-	-	-	FB	-	-	-	-
Iris spp.	L	-	-	F	-	-	L	-
Eleocharis palustris	-	-	-	-	F	-	-	-
Calopogon tuberosus	-	-	O	-	FB	-	-	-
Dulichium arundinaceum	-	-	-	-	-	-	-	L

Table S10. September 5, 2018 phenological surveys of co-occurring species with *Eleocharis geniculata*. The LPS (Long Point Spike-rush) points (collected in 2007) and *E. geniculata* 2014 (located in Pond 2) are historically known populations of *E. geniculata*. I only used LPS points locate in Pond 2 and Pond 9 for these surveys. Patch 1 is *E. geniculata* Patch 1 in 2018. Y = young leaves, I = increasing leaf size, L = leaves, FB = flower/flower bud, O = open flower, F = fruit, R = ripe fruits/seeds.

Species	*E. geniculata* 2014	Patch 1	LPS 9	LPS 10	LPS 12	LPS 15	LPS 17	LPS 24
Liatris cylindracea	-	FB	-	-	-	-	-	-
Solidago spp.	-	I	-	-	-	-	-	-
Poa spp.	-	FB	-	-	-	-	-	-
Panicum flexile	-	FB	-	-	-	-	-	-
Schenoplectus spp.	-	-	-	-	-	-	-	FB
Juniperus horizontalis	L	-	-	-	-	L	L	-
Juncus spp.	FB	FB	-	-	O	FB	FB	-
Triglocan spp.	F	-	F	FB	FB	-	Y	F
Eupatorium perfoliatum	L	-	L	-	-	-	-	-
Phragmites australis	-	L	FB	-	FB	-	-	-
Artemisia biennis	-	L	-	-	-	-	-	-
Equisetum spp.	-	FB	-	I	-	-	-	-
Carex aurea	-	R	-	-	-	-	-	-
Carex buxbaumii	-	R	FB	-	F	F	F	-

Carex pellita/Carex lasiocarpa	-	F	-	-	-	-	-	-
Unknown	L	L	L	L	FB	L	L	L
Melilotus albus	-	FB	-	-	-	-	-	-
Lathryus palustris	-	O	-	-	-	-	-	-
Thuja occidentalis/ Juniperus virginiana	-	F	-	L	R	-	-	-
Fragaria virginiana	-	L	-	-	L	-	-	-
Polygonum/ Potamageton spp.	-	L	-	L	-	-	-	-
Hypericum perforatum	I	I	Y	-	-	-	-	-
Castilleja coccinea	-	FB	-	-	FB	-	-	-
Sisyrinchium spp.	-	R	-	-	-	-	-	-
Medicago lupulina	-	O	-	-	-	-	-	-
Schoenoplectus pungens	-	-	O	L	-	-	-	-
Scirpus microcarpus	-	F	-	-	-	-	-	-
Rosa palustris	L	-	-	-	-	L	-	-
Triadenum fraseri	-	-	-	-	-	Y	-	-
Lysimachia thyrsiflora	-	-	-	L	L	-	-	L
Thelypteris palustris	L	-	L	-	-	L	L	-
Thypa spp.	-	-	-	I	-	-	-	-
Pontederia cordata	-	-	-	FB	-	-	-	-
Iris spp.	L	-	-	F	-	-	L	-

Eleocharis palustris	-	-	-	-	F	-	-	-
Calopogon tuberosus	-	-	O	-	FB	-	-	-
Dulichium arundinaceum	-	-	-	-	-	-	-	L

Table S11. September 28, 2018 phenological surveys of co-occurring species with *Eleocharis geniculata*. The LPS (Long Point Spike-rush) points (collected in 2007) and *E. geniculata* 2014 (located in Pond 2) are historically known populations of *E. geniculata*. I only used LPS points locate in Pond 2 and Pond 9 for these surveys. Patch 1 is *E. geniculata* Patch 1 in 2018. Y = young leaves, I = increasing leaf size, L =leaves, FB = flower/flower bud, O = open flower, F = fruit, R = ripe fruit/seeds, RD = recent fruits/seeds drop, U = unripe cone, S = senescing.

Species	*E. geniculata* 2014	Patch 1	LPS 9	LPS 10	LPS 12	LPS 15	LPS 17	LPS 24
Lobelia spp.	-	O	-	-	-	-	-	-
Solidago spp.	-	O	-	-	O	-	-	-
Panicum flexile	-	F	-	-	-	-	-	-
Sorghastrum nutans	-	F	-	-	RD	-	-	-
Juncus spp.	RD	-	RD	-	F	RD	RD	RD
Triglocan spp.	R	-	R	F	-	R	R	F
Aster spp.	-	O	O	-	O	-	FB	-
Eupatorium perfoliatum	-	-	O	-	-	-	-	-
Phragmites australis	-	-	-	-	R	-	-	-
Equisetum spp.	-	-	F	-	F	-	-	-
Carex aurea	-	-	-	-	R	-	-	-
Unknown spp.	S	-	-	-	-	S	S	-
Thuja occidentalis /Juniperus virginiana	-	-	-	-	U	-	-	-
Hypericum perforatum	-	-	F	-	-	-	-	-

Species	*E. geniculata* 2014	Patch 1	LPS 9	LPS 10	LPS 12	LPS 15	LPS 17	LPS 24
Schoenoplectus pungens	-	-	R	S	-	-	-	-
Rosa palustris	R	-	-	-	-	R	R	YL
Triadenum fraseri	-	-	-	-	-	-	-	R
Polygonum spp.	-	-	-	-	-	-	-	OF
Lysimachia thyrsiflora	-	-	-	S	-	-	-	S
Schoenoplectus acutus	-	-	-	-	-	-	-	S
Potamageton spp.	RD	-	-	S	-	RD	R/RD	-
Calamagrostis canadensis	RD	-	RD	-	-	R	RD	-
Juniperus horizontalis	L	-	-	-	-	L	L	-
Thypa spp.	-	-	-	S	-	-	-	-
Sagittaria spp.	-	-	-	S	-	-	-	-
Pontederia cordata	-	-	-	S	-	-	-	-
Gentianopsis crinite	-	-	-	-	O	-	-	-
Muhly spp.	-	-	-	-	F	-	-	-
Helenium autumnale	-	-	O	-	-	-	-	-
Parnassia palustris	-	O	-	-	-	-	-	-

Table S12. Soil macro- and micro-nutrients from three cores in Patch 1 and three cores in Patch 2 in 2018. The soil cores were analyzed at the University of Guelph Agriculture and Food Laboratory. The asterisk indicates that values were below the minimum level of quantification but above the detection limit for the method. In such cases, the limit of quantification is indicated.

Soil Sample	1	2	3	4	5	6
Total Carbon (% dry weight)	2.89	2.65	1.97	2.26	2.9	2.52
Inorganic Carbon (% dry weight)	1.88	2.16	1.61	1.87	2.15	2.06
Organic Carbon (% dry weight)	1.01	0.492	0.36	0.395	0.752	0.458
NH4-N (mg/kg dry)	16	1.42	3.61	0.451	2.27	1.29
NO3-N (mg/kg dry)	0.449	0.318	0.417	0.286	0.923	0.283
Soil moisture (%)	49.75	27.01	26.51	21.76	33.75	28.78
P (mg/L soil dry)	3.6	2.5	3.5	1.5	4	1.4
Mg (mg/L soil dry)	97	35	36	49	46	52
K (mg/L soil dry)	17	<10*	<10*	<10*	11	<10*
Na (mg/L soil dry)	33	20	25	27	23	21
Ca (mg/L soil dry)	3100	2700	2500	2700	2700	2800
Mn (mg/L soil dry)	12	12	8.7	9.4	15	12
Zn (mg/L soil dry)	1.4	0.69	0.56	0.36	0.31	0.18
Cu (mg/L soil dry)	0.42	0.66	0.2	0.26	0.27	0.26
Fe (mg/L soil dry)	64	21	30	12	24	16
pH (mg/L soil dry)	7.6	8	8.1	8.2	7.8	8.2