Mitigating the Impacts of Climate Change on Rare Plants through Assisted Migration

by

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Abstract

Given high levels of human disturbance and projected rates of climate change, many species will be unable to keep pace with their shifting climatic ranges and thus face increased risk of extinction. This research investigated the use of assisted migrations for two rare and range-restricted plant species in Alberta: *Houstonia longifolia* and *Liatris ligulistylis*. The reproductive ecology of the distylous species *Houstonia longifolia* was also investigated to better understand limitations in reproduction and to help develop more successful conservation strategies. Assisted migration trials for both *Houstonia longifolia* and *Liatris ligulistylis* involved the translocation of mature plants and seeds to replicate sites at each of four geographic locations along a north-south gradient in Alberta being both south (future warming) and north (future climatic habitat) of the species current range. *Houstonia longifolia* flower morph ratios were more pin biased in small populations with seed production varying by morph type and proportion of plants in the surrounding area with pin flower morphs. As the proportion of pins increased, seed production increased in thrums, while decreasing in pins. This demonstrates that *Houstonia longifolia* has a self-incompatibility system that requires the presence of opposite floral morphs for maximum seed production. The assisted migrations of *Houstonia longifolia* resulted in 6.2% of adult plant survival from mostly within its current range and no seed germination at any site. In contrast, *Liatris ligulistylis* adult plants and seed were able to grow and establish hundreds of kilometers outside of their current range. Results suggest that *Liatris ligulistylis* may be out of equilibrium with the climate in its current range with some measures of plant performance best 500 km north of its current range. The inclusion of soil from source locations also improved plant performance. Translocation of plants with source soil may therefore improve the success of these and future assisted migrations. It is hoped that through the continuation of these trials, assisted migrations will become a more recognized and utilized conservation strategy to prevent the loss of biodiversity.

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Chapter 1: General Introduction

1.1 Background

As climate change progresses, climatic niches of species will shift to higher latitudes and altitudes requiring the migration of species and movement of their ranges or *in situ* adaptation (Early and Sax, 2011; Burrows et al., 2011; Chauvenet et al., 2013). Given the predicted rates of climate change, many terrestrial and freshwater species will be unable to migrate at the pace necessary to maintain their current climate range (niche) (Atkins and Travis, 2010; Early and Sax, 2011; Sté-Marie et al., 2011; Schwartz et al., 2012). Plants (trees and herbaceous plants) in particular will face higher risks of extirpation or even extinction, as they have some of the lowest maximum migration rates of all taxa (Vitt et al., 2010; Field, 2014). This will be particularly true for those plant species with narrow climatic niches with high habitat-specificities.

Habitat loss and fragmentation are also posing threats to the persistence of many species and will become increasingly problematic for those species with limited dispersal abilities. Landscape connectivity promotes plant persistence, especially for those species with dispersal limitations (Matlack and Leu, 2007). Unlike animals, plant movement between habitats is limited by seed dispersal. Therefore, large spatial gaps can lead to extinctions if unsuitable sites for germination are encountered by all propagules of a single generation (Lavorel et al., 1995). To prevent extirpations and promote future colonization, connectivity between suitable habitats is therefore needed (Söndgerath and Schröder, 2002). However, maintaining this connectivity may be nearly impossible with increasing levels of human-disturbance.

For generations the use of protected areas, stepping stones and corridors have been accepted as conservation strategies to preserve biodiversity (Hannah, 2008; Hewitt et al., 2011). However, with the predicted rate of climate change and levels of fragmentation in many regions, new interventions will be increasingly necessary for reducing the risk of extinction for those vulnerable species. One potential tool is assisted migration (Sté-Marie et al., 2011), also referred to as managed relocation (Richardson et al., 2009; Schwartz et al., 2012) or assisted colonization (Hoegh-Guldberg et al., 2008; Seddon, 2010). This tool involves moving organisms to higher latitudes and altitudes which are predicted to be more suitable under future climate change scenarios (McLachlan et al., 2007; Loss et al., 2011).

There is currently much debate over the use of this conservation tool. One of the main concerns is that translocated species could become invasive and cause negative effects on the native flora and fauna at recipient sites (Riccardi and Simberloff, 2009). However, translocation of plants within the same continent, and into areas lacking local endemics, are unlikely to cause these negative effects (Mueller and Hellmann, 2008; Thomas, 2011).

There is also the concern of intervening in nature and creating novel ecosystems comprised of new plant assemblages (Seddon, 2010). Some feel we should not intervene in nature and allow it to take its own course of action. There are also those who feel we should intervene, but only to help restore areas to a pre-determined historical state (Seddon, 2010, Hobbs et al., 2011, Higgs et al., 2014). However, given the extent of habitat change in many regions, and the pace of climate change it will be increasingly difficult to restore landscapes back to their prior condition. Finally, there are those individuals who believe intervention is necessary in a world of increasing human disturbance, and the creation of novel ecosystems through the movement of plants will be necessary to preserve biodiversity for future generations (Hobbs et al., 2011).

Despite concerns regarding assisted migration, trials have already occurred for some species such as *Pinus albicaulis* Engelm (Whitebark pine) in British Columbia (McLane and Aitken, 2012), and for two butterfly species in the U.K. (Willis et al., 2009). One of the most publicized trials has involved the translocation of the conifer *Torreya taxifolia* from its current range in northern Florida to North Carolina in order to prevent its future extinction (www.torreyagardians.org). In Western North America the forest industry is also proactively experimenting with the movement of 16 tree species to northern latitudes under the Assisted Migration Adaption Trial Project (Marris, 2009; Williams and Dumroese, 2013). However, the number of experimental trials is still small (Hewitt et al., 2011). More trials for more species are needed as these experiments provide necessary empirical evidence that can be incorporated into decision-making frameworks and management protocols (Hoegh-Guldberg et al., 2008; Richardson et al., 2009; McDonald-Madden et al., 2011; Schwartz et al., 2012).

Species targeted for assisted migration should have low dispersal abilities, high habitat specificity, narrow climate niches, and occur in areas with high levels of habitat fragmentation (Hunter, 2007; Early and Sax, 2011; Loss et al., 2011; Sté-Marie et al., 2011). Both the long-leaved bluets (Rubiaceae: *Houstonia longifolia*) and northern blazing star (Asteraceae: *Liatris ligulistylis*) are species believed to possess these characteristics making them ideal candidates for testing the use of assisted migration as a conservation tool. These species are found in Alberta, this being their northwestern range limit, and both are restricted to the Central Parkland Natural Subregion (Alberta Conservation Information Management System [ACIMS], 2015). This area has experienced some of the highest levels of habitat loss and fragmentation in the province as a result of oil and gas exploration and extraction, grazing, and conversion of parkland to till cropping (Natural Regions Committee, 2006). As industry continues to expand in this area, and climate change progresses, numerous species will face an increased risk of extinction (Thomas et al., 2004).

Houstonia longifolia (Moss and Packer, 1983) is a perennial forb found in five Canadian provinces (Alberta, Saskatchewan, Manitoba, Ontario, and Quebec) and in mid-western and eastern USA having a globally 'secure' to 'apparently secure' conservation status of G4G5. Within Canada this species status ranges from 'secure' and 'apparently secure' within Saskatchewan (S4S5), Manitoba (S4S5) and Ontario (S4), to 'critically imperiled' within Quebec (S1). However, within Alberta where the species is at its north and west range limit, it is considered 'imperiled' (S2) (NatureServe, 2013). It is also ranked 10th most vulnerable to climate change out of 419 rare plants assessed in Alberta (Barber et al., 'in press'). Within Alberta this species is usually found on sandy woodlands and dunes (Royer and Dickenson, 2007), and its seeds lack any features designed to improve dispersal making it highly dispersal limited (Kershaw et al., 2001; Figure 1-1). *Houstonia longifolia* is distylous, with two morphs: pin (long-style) and thrum (short-style), and is believed to have a heteromorphic incompatibility system with crosses between thrums and pins being necessary for the production of seed (Beliveau and Wyatt, 1999). However, little is known about this species' reproductive ecology and the factors which promote its seed reproduction.

Liatris ligulistylis (Moss and Packer, 1983) is a perennial forb found in the midwestern USA and in three Canadian provinces (Alberta, Saskatchewan and Manitoba) having a globally 'secure' conservation status of G5. It is considered 'secure' (S5) in Saskatchewan and 'apparently secure' (S4) in Manitoba, but has a status of 'vulnerable' (S3) in Alberta where it too is at its northern and western range limit (ACIMS, 2015). In Alberta, this species is usually found on stable, isolated aeolian sand dunes and along sandy ditches (Pedersen, personal observation). Its seed morphology, with a pappus attached to the achene, is believed to only allow for short distance wind dispersal based on dispersal rates of *Liatris spicata* (COSEWIC, 2012; Figure 1-2). However, it is considered less dispersal limited than *Houstonia longifolia*.

1.2 Objectives

The goal of the research presented in this thesis was to develop an understanding of the biology of these rare and range-restricted species, and to incorporate this understanding into the development of assisted migration conservation strategies. Rare here refers to those species which are geographically restricted (i.e. within central parkland natural subregion), but within specific habitat locally abundant (Rabinowitz et al., 1986).

In Chapter 2, I investigate the reproductive ecology of *Houstonia longifolia* and explore conservation strategies for its persistence. I assessed the effect of population size on flower morph ratio, and examined the effects of density, morph type, population size, pin proportion, and maximum height on seed production. I then investigated which factors best predict seed production. Finally, I examined whether there are germination differences between morph types. Recommendations for the conservation of this species are then discussed, including methods for future assisted migrations.

In Chapter 3, I explore the success of assisted migrations as a conservation tool for *Houstonia longifolia* by establishing 13 translocation sites across Alberta along a latitudinal gradient from warm, dry prairies in the south (equivalent to future warming in its current range), to the central parklands (current range), to northern, boreal forests far to the north of its current range but anticipated to be equivalent to the current ranges climate in the future. Given this design, I assessed climatic vulnerabilities to future warming by comparing survival rates, growth (height), reproduction (flower production) and seedling establishment at southern sites to those at sites within this species' current range. These southern sites were set-up as they reflect the expected warming conditions for the 2080s in this species' current range. I then assessed the success of these assisted migrations by comparing measurements at the higher latitude sites to those sites within its current range.

In Chapter 4, I explore the success of assisted migration as a conservation tool for *Liatris ligulistylis* by establishing 12 translocation sites across Alberta along the same latitudinal gradient as the *Houstonia longifolia* sites. In this chapter I assessed the climatic vulnerabilities of this species to future warming by comparing survival rates, growth (height), reproduction (proportion bloomed, number of flower buds, seed production, proportion of full seeds), and seedling emergence/establishment levels south of this species' current range to those within its' current range. The southern sites again reflects the expected warming conditions for the 2080s in this species' current range. I then assessed the success of assisted migrations by comparting these above measurements at the higher latitudinal sites to those within this species' current range. To conclude this chapter I provided methods and protocols that could be incorporated into future trials.

In Chapter 5, I provide a synthesis of my work, including areas requiring future research and recommendations for how the results of this research can be incorporated into the future management of rare and range-restricted species in Alberta. This thesis was written following *Biological Conservation* journal guidelines and all chapters are unpublished.

1.3 Figures

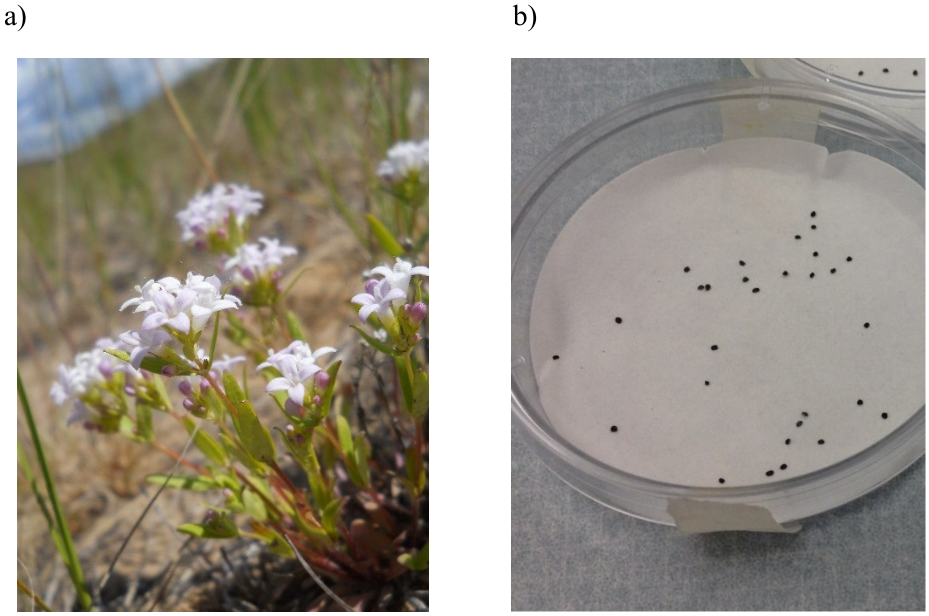


Figure 1-1: Adult long-leaved bluet (*Houstonia longifolia)* (a) (Photograph by S. Nielsen), and seed from central Alberta (b) (Photograph by J. Pedersen).

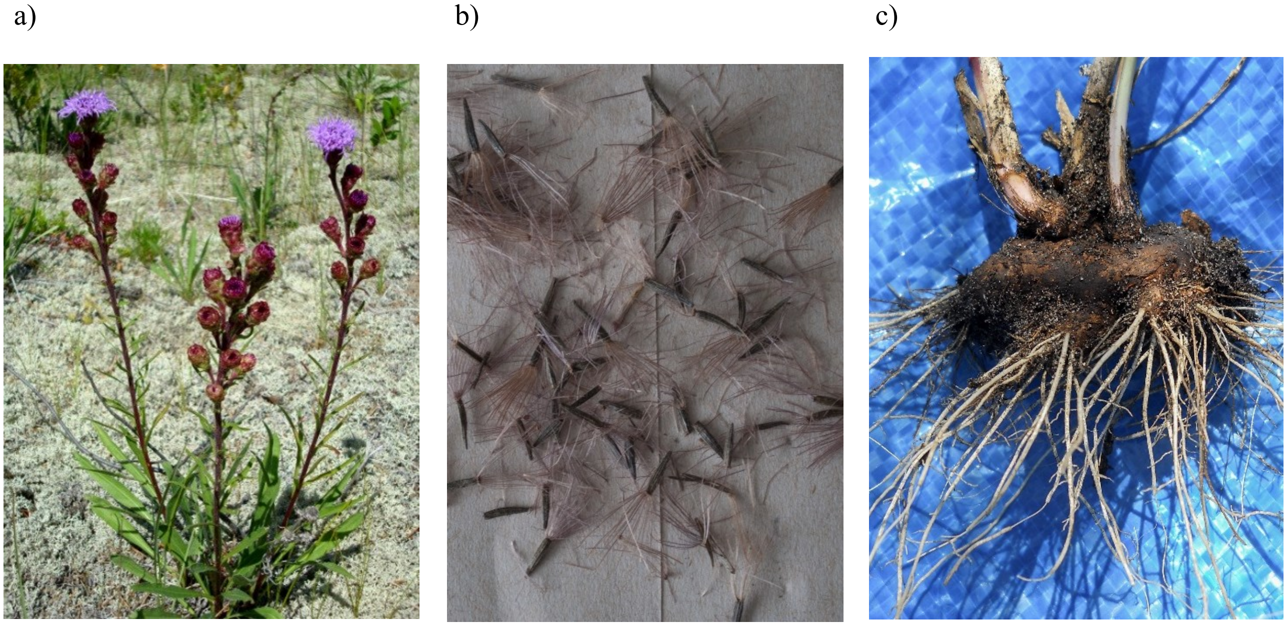


Figure 1-2: Adult northern Blazing star (*Liatris ligulistylis*) inflorescence (a), seed with pappus (b), and corm (c) from central Alberta (Photographs by S. Nielsen).

Chapter 2: Reproductive ecology of the distylous species *Houstonia longifolia*: Implications for conservation of a rare species

2.1 Introduction

Heterostyly is a type of sexual polymorphism found in 28 plant families (Barrett, 2002) where plants produce either two (distyly), or three floral morphs (tristyly) (Barrett, 2002). Distylous species exhibit herkogamy within flowers, with pins having long styles and short stamens and thrums having short styles and long stamens (Ganders 1979; Barrett, 2002). Thus in pin plants the stigma is positioned above the anthers while the reverse is true in thrum plants. Most distylous species also express heteromorphic incompatibility where only crosses between pins and thrums will lead to fertilization (Barrett, 2002). This incompatibility system is also believed to be maintained by the spatial separation of sexual structures in flowers (Barret, 2002), promoting the transfer of pollen from short stamens to short pistils and long stamens to long pistils by the segregation of pollen on different body segments of pollinators (Ganders, 1979; Meeus, 2012).

In distylous species pin morphs have been shown to be recessive homozygous (ss), with thrum morphs being dominant heterozygous (Ss) (e.g., *Primula veris*) (Van Rossum et al., 2006). Therefore, crosses between pins and thrums in distylous species are assumed to result in equal proportions of pin and thrum offspring, thus maintaining equal morph ratios (Van Rossum et al., 2006, Meeus, 2012). However, some distylous species have shown a breakdown of self-incompatibility (Ganders, 1979). This breakdown has been shown to occur most frequently for pin morphs (Ganders, 1979), although this can vary among morph types even within the same family. For example, in the family Rubiaceae, *Houstonia caerulea* thrums show lower self-incompatibility than pins (Wyatt and Hellwig, 1979), whereas the reverse occurs for *Houstonia nigricans* (Bahadur, 1970b; Levin, 1974). These breakdowns are believed to increase selfing and intramorph crosses leading to uneven morph ratios in populations experiencing a disruption in intermorph pollination (Ganders, 1979; Van Rossum et al., 2006; Meeus et al., 2012). For example, if pins (ss) are considered more self-compatible, then pin intramorph crosses (ss * ss) will lead to a pin bias in those populations experiencing a lack of compatible partners needed for intermorph crosses (Kery et al., 2003; Van Rossum et al., 2006). This selection away from even morph ratios is most likely to occur in small populations as they are more prone to “demographic stochasticity and genetic drift” (Molano-Flores, 2001; Endels et al., 2002; Kery et al., 2003; Van Rossum et al., 2006; Brys et al., 2008).

Studies have also shown that crosses between morphs (intermorph crosses) produce higher seed set than those between the same floral morph (intramorph crosses) (Nicholls, 1987; Beliveau and Wyatt, 1999; Shibayama and Kadono, 2003; Van Rossum et al., 2006). The maintenance of equal morph ratios is necessary for the continuation of these crosses, especially for those species with strong self-incompatibility. These intermorph crosses are also believed to retain more genetic diversity within a population, allowing species to respond to changing conditions (Kery et al., 2003; Shibayama and Kadono, 2003).

For distylous species, seed production differs among floral morph types. Pin morphs produce more seed than thrum morphs in some species (Beliveau and Wyatt, 1999; Matsumura and Washitani, 2000), while the opposite trend has also been observed for other species (Agren and Ericson, 1996; Minuto et al., 2014). These differences are thought to be attributed to the greater self-compatibility of one morph in areas experiencing a disruption in intermorph crosses, or perhaps by one morph being a “more efficient pollen recipient” than the other (Van Rossum et al., 2006; García-Robledo, 2008). Differences in seed viability between morph types have also been observed, with seed from pins usually having higher germination levels than seed from thrums (Nicholls, 1987; Rayburn et al., 2013).

Numerous biotic factors can also affect the seed production in distylous species. For instance, larger populations have been shown to have higher intermorph cross rates than small populations because these populations experience more even morph ratios and increased pollination levels (Kery et al., 2000; Matsumura and Washitani, 2000; Jacquemyn et al., 2002; Kery et al., 2003). Local floral density can also influence seed production (Feinsinger et al., 1991; Van Rossum et al., 2006), with smaller distances between morph types increasing the rate of intermorph crosses (Nicholls, 1987; Shibayama and Kadono, 2003). Past studies have also determined that seed set is positively related to the frequency of pins in a population (Van Rossum et al., 2006; Brys et al., 2008). This is likely due to thrums requiring pins for intermorph crosses, or perhaps pins being more self-compatible allowing for seed production even in the absence of thrums. However, no studies to my knowledge have investigated how the number of pins in close proximity to a focal plant of varying morph type affects seed production. Finally, plant height is also believed to be correlated with seed production – partly because taller plants have higher pollinator visitation rates (Schmitt, 1983; Brothers, 2014).

Therefore, there are multiple factors and morphological consideration which could potentially affect reproduction in distylous species. For this study the distylous species *Houstonia longifolia* (long-leaved bluet) was investigated in order to test and better understand which factors affect its reproductive success. The main objectives of this study were to: 1) determine if morph ratios differ from isoplethy in small populations, and whether these shifts are towards the pin morph, as this is predicted to be the more self-compatible morph; 2) examine how population size, local floral density, proportion pins surrounding a focal plants, plant height and morph type affect seed production; and 3) determine whether germination rates differ among the morph types for this species. Results of this study will inform conservation strategies by better understanding the reproductive ecology of *Houstonia longifiolia*.

2.2 Methods

2.2.1 Study species

The long-leaved bluets (*Houstonia longifolia* Gaertner, Moss and Packer, 1983) is a distylous perennial forb within the Madder family (Rubiaceae). This species has opposite leaves and purplish to white funnel-shaped flowers; each plant consists of multiple (1 to 100) short (~15 cm) reproductive stems. (Beliveau and Wyatt, 1999; Royer and Dickinson, 2007). *Houstonia longifolia* is found in five Canadian provinces (Alberta, Saskatchewan, Manitoba, Ontario, and Quebec) and across the midwest and eastern USA having a global ranking of G4G5 (NatureServe, 2013). The northwestern range limit of the species is in central Alberta where its conservation status is "imperiled" (S2) (NatureServe, 2013) and its distribution is restricted to the Central Parkland Natural Subregion (ACIMS, 2015). This species is found on sandy woodlands and dunes (Royer and Dickenson, 2007), and also along sandy roadside ditches (Pedersen, personal observation). It flowers from early June through July (Beliveau and Wyatt, 1999) with its seeds in Alberta maturing by September (Pedersen, personal observation). This species is also considered to be dispersal limited given that its seeds lack any features designed to improve dispersal (Kershaw et al., 2001).

2.2.2 Study area

The study was conducted within the Central Parkland Natural Subregion. This subregion has a mean annual temperature of 2.3°C, a mean annual precipitation of 441 mm, and 1412 growing degree days above 5°C (NRC, 2006). A mixture of deciduous forests and grasslands characterize this area. This region is also considered to be one of the most populated with some of the highest levels of habitat fragmentation and habitat loss in Alberta from oil and gas, grazing and agriculture (NRC, 2006). *Houstonia longifolia* populations found within this area were separated by areas of unsuitable habitat, such as forests, wetlands, roadways, and agricultural land.

2.2.3 Focal plant surveys

A pre-survey investigation occurred before the commencement of field work. This pre-survey investigation included: review of *Houstonia longifolia* phenological characteristics, Alberta Conservation Information Management System (ACIMS) occurrence records, consultations with Alberta Native Plant Council (ANPC) members, and searches of suitable sandy habitat using aerial imagery from Google Earth. On this basis a list of sites was generated for survey visits. Meander searches were then used at each site to identify locations with *Houstonia longifolia* and the extent of detected populations (following survey methods described in Lancaster, 2000; Henderson 2009). Targeted surveys were then undertaken at these sites from late June to Mid-July 2014 during the *Houstonia longifolia* flowering period. From these surveys a total of 14 *Houstonia longifolia* populations were found within the Central Parkland Natural Subregion (Table 2-1). Following these targeted surveys, random meander surveys were conducted at each location to locate focal plants (Henderson, 2009). Depending on population size, between 6 and 26 individuals were marked at each of the 14 populations, with a total of 210 individual *Houstonia longifolia* plants being used in this study. Focal plants were chosen based on varying levels of *Houstonia longifolia* plants surrounding them with multiple plants of each morph type being identified for study.

At each location chosen plants were marked with a flag and identification numbers given to indicate site location and morph type. A nail was also placed at the base of each plant and marked with its floral morph as a back-up. Although identification of pin and thrum floral morphs could be determined by the naked eye, magnifying lenses were also used. For each marked plant, GPS coordinates were recorded along with the maximum and mean (based on 10 stems) stem height.

2.2.4 Local density

A 1 m^2 quadrat was placed around each focal plant and used to quantify the number and proportion of pins and thrums surrounding each focal plant. Some plants surrounding the focal plant lost their flowers before morph identification could occur. These individuals were recorded as unknown and included in the total density, but not in calculations of the proportion of pins. The effect of pins surrounding the focal plant was therefore based on a subset of observations ($n = 185$ quadrats).

2.2.5 Population size

In July 2014 transect searches (100 m parallel transects bisecting populations) were used in each of the 14 known populations to estimate population size (Henderson, 2009). This type of search decreased the chances of overlap and increased the amount of area covered.

Search intensity at each location varied with the size of the population and visibility of *Houstonia longifolia* (Henderson 2009; Lancaster, 2000). For each transect, a 1 m^2 circular quadrat was systematically spaced every two to five meters, depending on the terrain and extent of population. In each quadrat the number of individuals present was recorded. Since plants were clumped spatially, individual counts were based on number of rosettes of basal leaves. Distances between transects varied from 3 to 20 meters, again based on terrain and extent of population (Table 2-1). Only one survey occurred for each population.

Total area (m^2) of each population was estimated by surveying the extent of the population by walking its perimeter using the TRACK function on a handheld GPS (Garmin Oregon 550). ArcGIS 10.2 was used to determine the total area (m^2) of each polygon (population) by using track data and the POLYLINE TO POLYGON and the CALCULATOR functions. For those areas where extents of population were not walked due to rough terrain, the beginning and end points of all line transects were used in the POINTS TO POLYGON to buffer around all lines within a population. From this area the CALCULATOR function was used to determine extent area (m^2).

Total number of individuals in all quadrats was divided by the total number of 1 m^2 circular quadrats to estimate average *Houstonia longifolia* density for each population. This average density (per 1 m^2) was then multiplied by the areal extent (total area m^2) of each population to estimate total *Houstonia longifolia* population size (Table 2-1).

2.2.6 Seed production

In September 2014 all 14 locations were visited and the focal plants were collected just prior to seed dispersal and full senescence of plants. Stems were cut at the base of the plant near the basal leaves, and placed into labeled coin envelopes. Due to the small size of seeds (< 1 mm), coin envelope flaps were taped and then placed into plastic bags to prevent seed loss during transport. Plastic bags were removed after transport to the lab, and any seeds contained in these bags were transferred to a new coin envelope labeled with the same ID. Coin envelopes were then put in brown paper bags and placed in window sills at the University of Alberta to promote further drying and seed ripening.

Of the 210 samples, 15 seed envelopes developed some mold. These were transferred to new envelopes, appropriately labeled and placed in a drying oven at 60°C for 48 hours. This drying prevented further growth of mold. Seeds from these envelopes were counted to estimate seed production but were not included in the germination tests.

Due to their small size, seeds for each marked plant were manually counted. For each plant, the number of stems was counted and any unopened capsules containing seeds were opened with tweezers. Magnifying lamps were used to assist with seed counting. After seeds were counted they were stored in a fridge to simulate a natural period of cold stratification. To ensure accuracy of seed counts, 6% of the plants were double counted with an average error rate of 3.97% (Appendix 2-1).

2.2.7 Germination

Germination rates were determined in the lab for one pin and one thrum chosen randomly from each of 12 populations. For each individual pin and thrum chosen, 30 seeds were placed on moistened (distilled water) filter paper in each of three petri dishes; these were placed in a window sill for natural light, as germination for this species is known to be induced by light (Baskin and Baskin, 1998). In total, there were 72 samples. The experiment lasted 40 days with daily seed moistening and monitoring of percent germination in each dish.

2.3 Analysis

The statistical software package R 3.1.2 was used in all analyses (R Core Team, 2014).

2.3.1 Morph ratio

Morph ratio was calculated as: (# Pins - # Thrum) / (# Pins + # Thrums) (Meeus et al., 2012). A morph bias of -1 indicates populations of only thrums and +1 indicates populations of only pins, with 0 indicating equal morph proportions (Meeus et al., 2012). A linear regression model using the stats package (R Core Team, 2014) was then used to determine the effect of population size (log10 transformed) on morph bias.

2.3.2 Influential factors

Univariate negative binomial generalized linear mixed-effects models using the lme4 package (Bates et al., 2014) were used to assess the effect of population size, local density, morph type, surrounding pin proportion, and stem maximum height on seed production of sampled plants. The negative binomial model was used because a Poisson model was determined to be over dispersed ($\varphi > 4$). For these models the number of stems per plant was added as a covariate, as well as morph type, with location included as a random effect. Logarithmic transformation of continuous predictors, except proportion pin, was used to improve model convergence. Proportions of 0 and 1.0 were replaced in the dataset with 0.001 and 0.999, respectively and then Arcsine transformed prior to testing the effect of the proportion of surrounding pins on seed production. Residuals for all models were checked for normality and homoscedasticity, with outliers (one observation) being removed for the local density model (n=209). With the lme4 package, significance (p-values) of factors are not provided and instead were calculated using Parametric Bootstraps set at 4999 iterations using the afex package (Singmann et al., 2015).

2.3.3 Candidate models for seed production

Regardless of significance of individual factors, I wanted to identify the set of factors that best predict seed reproduction in *Houstonia longifolia* (Table 2-2). Therefore, 31 candidate models were developed to examine which set of independent variables best explained seed production. These models included seed production (# of seeds per plant) as a response, location as a random factor, number of stems as a covariate and combinations of the following factors: population size, local density, proportion pin, morph type and maximum height of the focal plant. All possible combinations of these variables were run as it was hypothesized that all would be of importance to this species' reproductive success. The interaction between morph type and proportion pin was also included for all models that contained these variables, due to the prediction that seed production would vary with morph type of the focal plant and the proportion of pins surrounding it. For these models all continuous factors were log transformed to improve convergence of models, except for the variable proportion pin that was Arcsine transformed. In total, there were 210 observations for all models.

AIC was used to identify the most parsimonious model (Akaike, 1974) among the 31 candidate models. A Chi-square test was used to determine if the most supported model differed significantly from a null ecological model that contained only number of stems as a covariate and location as a random effect. For the most supported model the correlation between observed seed production and predicted values was calculated. Finally, to examine the nature of the influence of predictor variables on seed production, predicted values were graphed for the most supported model. Observed values were used for the factors graphed, while all other factors were held constant at their means.

2.3.4 Germination

A linear mixed effect model within the nlme package (Pinheiro et al., 2015) was used to test differences in germination rates between morph types. Complete germination rates (100%) did not occur in any of the petri-dishes, but there were cases of 0% germination. Germination percentages were divided by 100 and all proportions of 0 were replaced with 0.001 in the dataset for analysis. An Arcsine transformation of proportion germination rate was used as the response variable, with morph as a fixed effect and the three replicate petri-dishes nested in location as a random effect. Residuals were checked for normality and homoscedasticity. The lsmeans package (Lenth and Hervé, 2015) was used to calculate average germination proportion for each morph type.

2.4 Results

Morph ratios ranged from -0.21 (more thrum than pin) to 0.48 (more pin than thrum) across the 14 populations with population size significantly related to morph bias ($p = 0.005$, $R^2 = 0.49$). Small populations experienced a greater pin bias while large populations were thrum biased (Table 2-3 and 2-4, Figure 2-1). Population size, local density, morph type and proportion pin had no significant effect on seed production, although plant size (maximum stem height) was positively related to seed production (Table 2-5).

The most supported AIC model for seed production of *Houstonia longifolia* included plant height, morph type, proportion pin, number of stems as a covariate and location as a random effect ($AIC_{M25} = 3128.2$; Table 2.6). When compared to the null ecological model (M1) containing only number of stems as the covariate and location as the random effect, the most supported model (Model M25) was 2,421,748 times more supported (Akaike weights) than the null ecological model and was also significantly better than the null ecological model (χ^2 = 37.35, p <0.001) (Table 2-6). Within this model (Model M25) plant height, and the interaction between morph type and proportion pin were positively related to seed production, with the terms thrum morph and proportion pin were negatively related to seed production (Table 2-7). Seed production increased for thrums as number of pins surrounding it increased, while seed production in pins decreased as the proportion of pins surrounding it increased (Figure 2-2). The correlation of observed values to predicted values for the most supported model (Model M25) was moderate in strength (r = 0.53), suggesting the presence of other unmeasured factors affecting seed production.

Germination rates differed among morph types of *Houstonia longifolia*, with germination rates for seed from the pin morph (46.7%) being significantly higher than seed from the thrum morph (32.3 %) (Tables 2-8 and 2-9).

2.5 Discussion

This study demonstrated that small populations of *Houstonia longifolia* were more pin biased while large populations tended to be more even to thrum biased. As expected, taller plants had higher seed production than short plants, and germination rates of seed from pins were higher than those in thrums. Based on our most supported model, seed production is predicted to vary with morph type and proportion pin, with increases in proportion of pin related to declines in seed production for pins but increases in seed production for thrums.

Large populations of distylous species typically have even morph ratios, with reductions in population size leading to a morph bias towards the more self-compatible morph (Molano-Flores, 2000; Endels et al., 2002; Kery et al., 2003). With most distylous species experiencing a lower self-incompatibility in pins (*Hedyotis nigricans*, *Pentas lanceloata*, *Pulmonaria officinalis*, *Jepsonia heterandra*, and *Primula sieboldii*) (Bahadur, 1970b; Bahadur 1970a; Ornduff, 1971; Matsumura and Washitani, 2000; Brys et al., 2008), it was hypothesized that *Houstonia longifolia* pins would also have a higher self-compatibility, leading to pin morph biases in small populations, which were believed to be more susceptible to morph losses from stochastic events (Endels et al., 2002; Jacquemyn et al., 2002; Kery et al., 2003; Brys et al., 2008). The relationship between morph bias and population size did conform to expectations, with small populations (< 73 individuals) being more pin biased and large populations being more thrum biased. The pin floral morph therefore may have greater self-compatibility than the thrum floral morph. However, due to the short term nature of this study, future research is needed to determine the strength of this self-compatibility.

It was hypothesized that increases in population size would lead to increases in seed production, due to larger populations having more even morph ratios and also being more attractive to pollinators (Jacquemyn et al., 2002; Kery et al., 2000; Matsumura and Washitani, 2000; Kery et al., 2003). However, differences in population size for *Houstonia longifolia* were found to have no significant effect on seed production. There are several possible explanations for this result. First, with the small stature of this species even large populations may not have been differentially attractive to pollinators, which could have led to similar pollination rates regardless of population size. Second, cost of movement of pollinators between plants may affect visitation rates with greater visitation rate per plant in small populations (Waites and Agren, 2004). This could lead to a decline in pollen transfer and seed production for individual plants in large populations. Finally, these populations may also experience a phenomenon known as "ideal free distribution" (Fretwell and Lucas, 1969) where pollination rates for each flower are equal regardless of population level or plant size (i.e. number of flowers) (Dreisig, 1995; Robertson and MacNair, 1995).

This "ideal free distribution" may also explain the lack of effect of local density on seed production. A paper by Dreisig (1995), who regarded plants of many open flowers as patches, found visitation rates to be equal for every flower regardless of the number of open flowers (i.e. patch size). The pollination of *Houstonia longifolia* flowers within a patch of multiple individuals may have also been even, regardless of the surrounding local density of open flowers. No relationship between seed production and local density was found for other plant species, including *Agalinis strictifolia* and *Arcytophyllum lavarum* (Dieringer, 1991; García-Robledo and Mora, 2007). However, other studies have shown intermorph pollination to be positively related to plant density (Schaal, 1978; Nicholls 1987; Shibayama and Kadono, 2003).

Based on previous studies, it was hypothesized that seed production would be positively related to the proportion of pins and that it would also vary between morph types (Shibayama and Kadono, 2003; Van Rossum et al., 2006; Brys et al., 2008). For example, in an experiment with the distylous species *Primulonaria officinalis,* fecundity in thrums was discovered to be positively related to pin morph frequencies (Brys et al., 2008). Van Rossum et al. (2006) also found seed set for *Primula veris* to be positively related to increases in proportion pins and to vary among morph types. Although no significant differences in *Houstonia longifolia* seed production were found between pins and thrums, the most supported predictive model indicated that seed production varied with morph type as well as the proportion of surrounding pins.

Increases in proportion of pins are predicted to be positively related to seed production in thrums, and negatively related to seed production in pins. This provides support for this species' self-incompatibility and how the occurrence of compatible mates will improve seed production through intermorph crosses. This also indicates that if pin intramorph crosses are to occur as pin proportions increase, seed production for this species would most likely decline.

From this study, plant height was positively related to seed production. This effect is most likely due to the increased fitness and robustness associated with taller plants, which were more likely to have more flowers. Unfortunately, flower data were not collected due to the short flowering period causing a continual loss of flowers during the duration of these surveys. This increase in seed production for tall plants may have been related to pollination levels. Schmitt (1983) found stalk height to affect pollinator selection and Levin and Kerster (1973) discovered pollinators to have "economic foraging" strategies resulting in the selection of similar height plants. This pollination strategy and selection was also found for the dioecious *Silene latifolia*, where visit rates were greater for tall plants (Brothers, 2014). Therefore, future studies should investigate if pollinator visits vary with *Houstonia longifolia* stem height.

Germinations levels were also found to vary between morph types, with seed from pins having significantly higher germination than thrums. Nicholls (1986) also found differences in germination between flower morphs in *Lythrum salicaria* with mid-style morphs having higher germination levels than the short style morph. Likewise, Rayburn et al. (2013) found that seeds from the pin morph for the distylous primrose *Primula cusickiana* had a higher germination rate than seed from the thrum morph. However, it is still unknown what mechanism dictates these differences. Future research is needed to investigate the factors affecting this species' seed germination.

2.5.1 Implications for conservation

Based on the results of this study, smaller populations had greater biases in pin morphs. Large population sizes should therefore be maintained since morph biases could reduce seed production (decrease in intermorph crosses) and thus potential local persistence of populations. In extreme cases, translocation of the less abundant morphs into small populations may need to occur or hand pollination to promote the continuation of intermorph crosses (Agren and Ericson, 1996).

Climate change may also affect the persistence of existing populations given that *Houstonia longifolia* is highly dispersal limited, habitat specific, and located within a narrow climatic range (Early and Sax, 2011; Ste-Marie et al., 2011). Assisted migration may be considered as a conservation tool to mitigate future declines of this 'imperiled' species (McLachlan et al., 2007; Vitt et al., 2010). Although tall plants produced a greater amount of seed, stem height is likely to vary between years. Therefore, if translocations to higher latitudes were to occur focus should be on placing equal proportions of pins and thrum at recipient sites in order to increase the chances of intermorph crosses. However, to determine the potential success of these methods, assisted migration trials with monitoring are needed.

2.5.2 Summary

Small populations of *Houstonia longifolia* in the Central Parkland were more pin morph biased. Seed production was positively related to plant height, and varies with morph type and surrounding proportion of pins. These results could be explained by pollination interactions, such as the asymmetric transfer of pollen between morph types and potential pollen limitations (i.e. intermorph pollen disruptions). Therefore, future research should investigate the abundance of pollinators and their transfer of pollen in populations of different sizes, local densities and proportion pin morphs. Also, further research should focus on determining additional factors which may improve our understanding of seed production in this species.

With this study and future research, there will be greater understanding of *Houstonia longifolia's* reproductive ecology, allowing for the formulation of appropriate conservation tactics. Biology should dictate conservation strategy, and therefore understanding the biology of other imperiled species will promote their persistence and survival on the landscape.

2.6 Tables

Table 2-1: Summary of *Houstonia longifolia* populations and sampling methods. Population size estimates (95% CI) were calculated by multiplying average density (# plants/m^2) by total habitat area (m^2).

Locations	Latitude	Longitude	# of Transects	Spacing between Transects (m)	Spacing between quadrats (m)	Average Density (# plants/m^2)	Total Area (m^2)	Population Estimates
Gibbons Field (Private Property)	confidential		20	10	5	1.23 (SE=0.45)	40,000.0	49200 (+/-35,280)
Gibbons Dune	53.863	113.319	9	15	5	0.55 (SE=0.46)	10,000.0	5500 (+/-9,016)
North Bruderheim Provincial Recreation Area	53.856	112.934	82	20	2	0.02 (SE=0.03)	200,180.5	4004 (+/-3,924)
West Bruderheim (1)	53.865	112.928	18	5 (small areas) or 10 (large areas)	2	0.16 (SE=0.11)	19,514.1	3122 (+/-4,207)
Northwest of Bruderheim Natural Area	53.850	113.029	25	4	2	0.12 (SE=0.07)	13,327.2	1599 (+/-1,828)
East Bruderheim (2)	53.867	112.923	27	5 (small areas) or 10 (large areas)	2	0.06 (SE=0.03)	16,809.9	1009 (+/-988)
Pipeline clearing near Andrew, AB	53.981	112.759	20	5	2	0.07 (SE=0.02)	11,800.0	826 (+/-462)
Lily Lake Natural Area	53.949	113.372	7	4	2	0.16 (SE=0.12)	3,746.0	599 (+/-881)
Fort Saskatchewan Prairie	53.681	113.270	20	15	2	0.02 (SE=0.01)	27,193.0	544 (+/-532)
Bellis Lake Natural Area	54.115	112.173	52	5 (small areas) or 10 (large areas)	2	0.02 (SE=0.00)	26,983.0	540 (+/-528)
Ditches near Smokey lake	54.046	112.357	6	3	2	0.19 (SE=0.12)	1,145.6	218 (+/-269)
Property near Bruderheim	confidential		3	3	2	0.45 (SE-0.36)	162.8	73 (+/-114)
Berm of Wellsite	53.833	113.052	6	3	2	0.02 (SE=0.01)	1,121.0	22 (+/-21)
Railway Park	53.811	113.051	5	3	2	0.08 (SE=0.04)	272.3	22 (+/-21)

(1) Section of North Bruderheim Provincial Recreational Area on west side of road

(2) Section of North Bruderheim Provincial Recreational Area on east side of road

Table 2-2: Summary of all independent variables tested in the 31 candidate models, along with their predicted responses on seed production.

Variables	Definition	Predicted Response
Population Size	Estimated population size of *Houstonia longifolia* for each of the 14 populations	Population size was predicted to be positively related to seed production
Local Density	Number of pins and thrums contained within a 1 m^2 quadrat surrounding the focal plant	Local density was predicted to be positively related to seed production
Maximum Height	Maximum height of focal *Houstonia longifolia* plants	Increases in maximum height were predicted to be positively related to seed production
Morph Type	Whether the floral morph of the focal plant was a pin (long-style) or thrum (short-style)	Differences in seed production between morph types will occur. Pins predicted to produce significantly more seed than thrums
Proportion Pin	Proportion of pins surrounding the focal plant within a 1 m^2 quadrat	Increases in the proportion of pins were predicted to lead to be positively related to seed production of focal plants

Table 2-3: Mean (and 95% confidence interval) morph bias (Pin-Thrum/ (Pin+ Thrum)) values for each location ordered by population size. Morph bias values ranged from -1 (all thrums) to +1 (all pins) with 0 indicating an equal morph ratio.

Location	Population size	Morph Bias	Lower CL	Upper CL
Gibbons Field	49200	-0.12	-0.43	0.18
Gibbons Dune	5500	-0.21	-0.50	0.07
North Bruderheim Provincial Recreation Area	4004	-0.15	-0.42	0.11
West Bruderheim	3122	-0.13	-0.41	0.16
Northwest of Bruderheim Natural Area	1599	0.19	-0.10	0.48
East Bruderheim	1009	0.01	-0.38	0.39
Pipeline clearing near Andrew, AB	826	-0.04	-0.50	0.41
Lily Lake Natural Area	599	-0.02	-0.71	0.67
Fort Saskatchewan Prairie	544	0.02	-0.37	0.42
Bellis Lake Natural Area	540	-0.23	-0.51	0.04
Ditches near Smokey lake	218	-0.11	-0.48	0.26
Property near Bruderheim	73	0.45	-0.11	1.01
Berm of Wellsite	22	0.20	-0.42	0.82
Railway Park	22	0.48	-0.04	1.00

Table 2-4: Results of linear mixed effects model testing the effect of population size (log10 transformed) on morph bias, with location as a random effect.

	β	SE	F	p
Log10(Population)	-0.17	0.05	11.92	0.005

Table 2-5: Results of negative binomial generalized linear mixed-effects models testing for the effects of population size, local density, morph type, stem max height, and proportion of pins on seed production in *Houstonia longifolia*. The number of stems and morph type for each focal plant were included as covariates in all models, with location set as a random effect. All continuous predictor values, except proportion pin (Arcsine transformation), were log transformed to improve model convergence. The p-values and stat coefficients were determined by means of parametric bootstraps (4999 iterations). p ≤0.05 '*'

Model	Effect	β	stat	p
Population	log(Population)	0.04	0.20	0.680
	log(Stems)	0.70	52.45	<0.001*
	Morph	-0.10	0.06	0.810
Density	log(Density +1)	0.105	2.84	0.100
	log(Stems)	0.743	56.90	<0.001*
	Morph	-0.06	0.02	0.900
Morph Type	Morph	-0.10	0.05	0.830
	log(Stems)	0.72	54.50	<0.001
Stem Max Height	log(Max Height)	0.82	4.19	0.050*
	log(Stems)	0.68	45.03	<0.001*
	Morph	-0.18	0.28	0.600
Proportion Pin	log(Prop.Pin +1)	0.01	0.21	0.650
	log(Stems)	0.72	44.21	<0.001*
	Morph	0.05	0.81	0.370

Table 2-6: Summary of 31 candidate negative binomial generalized linear mixed-effects models used to predict seed production in *Houstonia longifolia*. Model M25 in bold was the most supported model (lowest AIC).

Model ID	Model Structure	Log-likelihood	Model Complexity (K)	AIC	Delta AIC (ΔAIC)	Akiake weight (w_i)
1	L+S	-1574.8	3	3157.6	29.4	1.76E-07
2	L+S+N	-1574.4	4	3158.7	30.5	1.02E-07
3	L+S+D	-1572.7	4	3155.5	27.3	5.04E-07
4	L+S+H	-1569.7	4	3149.3	21.1	1.12E-05
5	L+S+M	-1574.5	4	3158.9	30.7	9.20E-08
6	L+S+P	-1574.1	4	3158.1	29.9	1.37E-07
7	L+S+N+D	-1572.7	5	3157.4	29.2	1.95E-07
8	L+S+N+H	-1567.7	5	3147.4	19.2	2.89E-05
9	L+S+N+M	-1574.0	5	3160.1	31.9	5.05E-08
10	L+S+N+P	-1573.7	5	3159.5	31.3	6.82E-08
11	L+S+D+H	-1564.2	5	3140.3	12.1	1.01E-03
12	L+S+D+M	-1572.6	5	3157.3	29.1	2.05E-07
13	L+S+D+P	-1572.0	5	3156.0	27.8	3.92E-07
14	L+S+H+M	-1568.5	5	3149.0	20.8	1.30E-05
15	L+S+H+P	-1569.3	5	3150.6	22.4	5.84E-06
16	L+S+M*P	-1565.0	5	3143.9	15.7	1.66E-04
17	L+S+N+D+H	-1563.4	6	3140.9	12.7	7.46E-04
18	L+S+N+D+M	-1572.6	6	3159.2	31.0	7.92E-08
19	L+S+N+D+P	-1572.0	6	3158.0	29.8	1.44E-07
20	L+S+D+H+M	-1563.5	6	3141.0	12.8	7.10E-04
21	L+S+D+H+P	-1563.9	6	3141.9	13.7	4.52E-04
22	L+S+N+H+P	-1567.6	6	3149.1	20.9	1.24E-05
23	L+S+N+M*P	-1565.0	6	3145.9	17.7	6.12E-05
24	L+S+N+H+M	-1566.3	6	3146.7	18.5	4.10E-05
25	**L+S+H+M*P**	**-1556.1**	**6**	**3128.2**	**0.0**	**4.27E-01**
26	L+S+N+D+H+M	-1562.6	7	3141.1	12.9	6.75E-04
27	L+S+N+H+M*P	-1555.4	7	3128.9	0.7	3.01E-01
28	L+S+N+D+M*P	-1564.6	7	3147.2	19.0	3.20E-05
29	L+S+N+D+H+P	-1563.3	7	3142.6	14.4	3.19E-04
30	L+S+D+H+M*P	-1556.1	7	3130.2	2.0	1.57E-01
31	L+S+N+D+H+M*P	-1555.4	8	3130.9	2.7	1.11E-01

L=location (random)
S=# of stems
N=population size
D=density
H=maximum plant height
M=morph
P=proportion pin

Table 2-7: Summary of model parameters for the most supported model (Model M25 in Table 2-6) of seed production in *Houstonia longifolia*. Model estimates for number of stems, maximum stem height, morph type (with β referring to thrums), proportion pin, and the interaction between morph and proportion pin interaction. Number of stems was included as a covariate with location as a random effect.

Fixed Effect	β	SE
log(Stems)	0.752	0.080
log(Maximum Height)	0.972	0.223
Morph (Thrum)	-1.233	0.245
Proportion Pin	-1.395	0.297
Morph (Thrum) : Proportion Pin	2.066	0.454

Table 2-8: Summary of germination rates for each morph type in *Houstonia longifolia*.

Morph	Germination Proportion	Lower CL	Upper CL
Pin	0.467	0.263	0.671
Thrum	0.323	0.118	0.527

Table 2-9: Linear mixed effect model results testing the differences in germination rates between morph types, with location as a random effect.

	num DF	den DF	F	p
Morph	1	35	15.02	<0.001

2.7 Figures

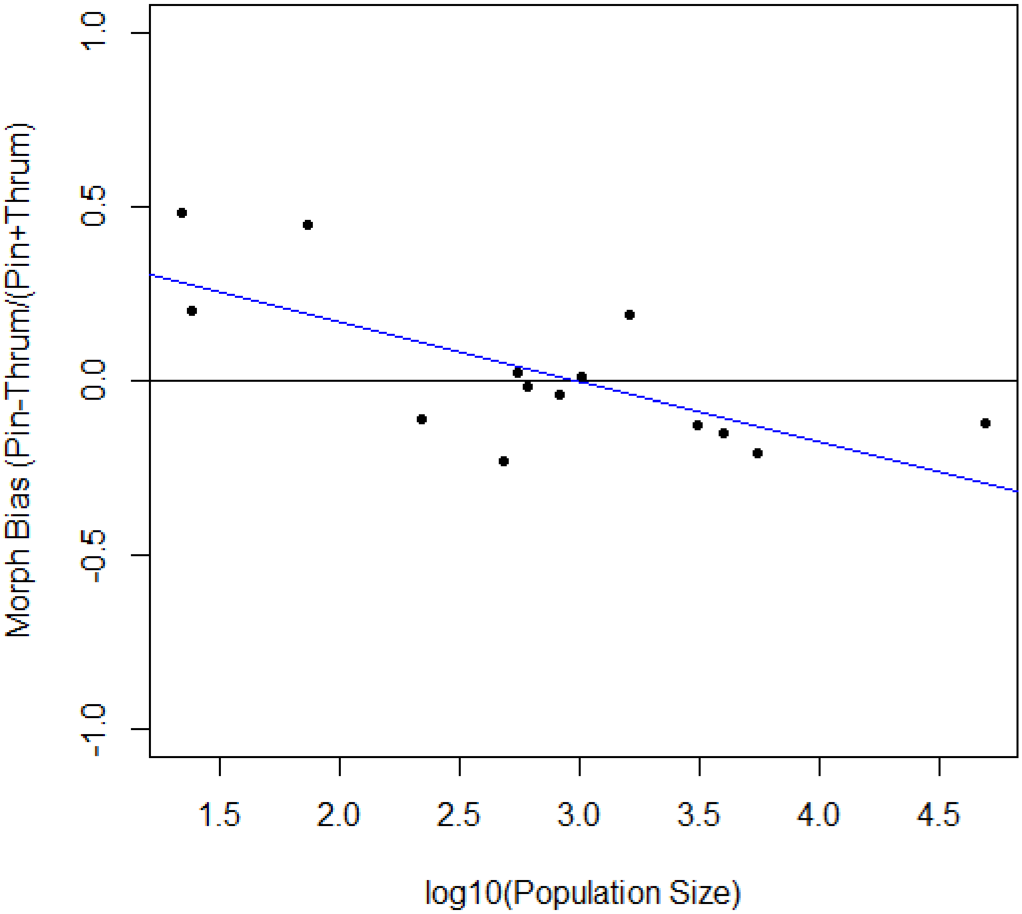


Figure 2-1: Average morph bias in *Houstonia longifolia* by population size. Morph bias varies between -1.0 (only thrum morphs) to 1.0 (only pin morphs). Isoplethy is indicated by the horizontal line at 0.0. The mean (linear regression) relationship between morph bias and population size is shown by the blue line. Some values are staggered for ease of interpretation.

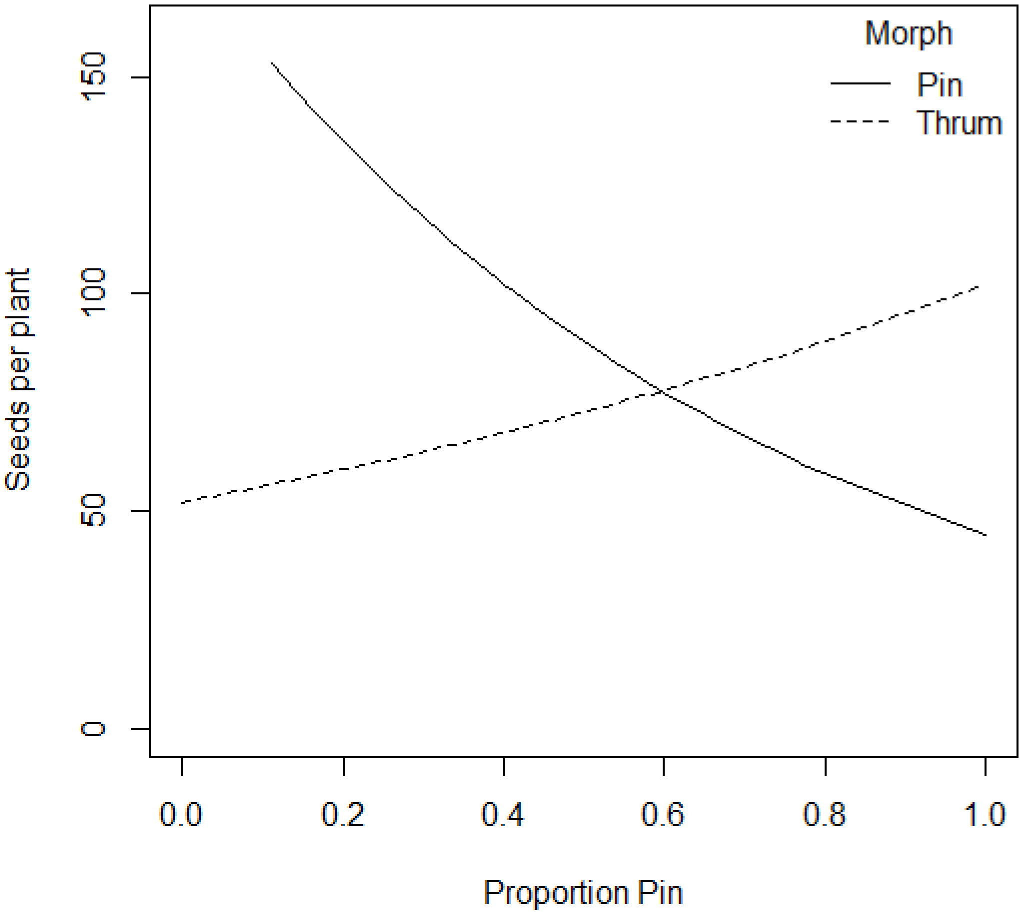


Figure 2-2: The relationship between proportion of surrounding pins and seed production in *Houstonia longifolia* for plants of each morph type. Predictions based on Model M25 with all other factors held at their mean.

2.8 Appendices

Appendix 2-1: Re-count data for *Houstonia longifolia* seeds. Percent Error refers to the difference between the first (Count 1) and second (Count 2) counts.

Plant ID	Count 1	Count 2	Percent Error (%)
Bluet G21	374	390	4.10
Bluet BE10	145	165	12.12
Bluet A9	676	656	3.05
Bluet FS8	837	820	2.07
Bluet SL9	324	334	2.99
Bluet DP4	204	205	0.49
Bluet DP1	78	78	0.00
Bluet 04	322	326	1.23
Bluet GD1	506	495	2.22
Bluet B12	815	805	1.24
Bluet G16	73	73	0.00
Bluet GD14	257	278	7.55
Bluet GD21	235	275	14.55
Average			3.97 (SE=1.29)

Chapter 3: An Assisted Migration trial for a rare and range-restricted species in Alberta: *Houstonia longifolia*

3.1 Introduction

Biodiversity is threatened by development and the quickening pace of climate change (Thomas et al, 2004, Schwartz et al. 2012). As climate change progresses, species' climatic ranges will shift to higher latitudes and altitudes (Parmesan, 2006; Burrows et al., 2011; Mahlstein et al., 2013; Dickinson et al., 2014). Those species with dispersal limitations, narrow climatic niches, high habitat specificity, and which occur within fragmented landscapes will be the most vulnerable to climate change (Early and Sax, 2011; Ste-Marie et al. 2011; Gallagher et al., 2015).

Assisted migration has been proposed as a proactive conservation strategy to prevent the loss of these species (McLachlan et al., 2007; Vitt et al., 2010; Schwartz et al., 2012). This tool involves the movement of species from their current range to areas expected to be more suitable under future climate change projections (McLachlan et al., 2007; Loss et al., 2011). The main concern over application of this conservation tool is that translocated species may become invasive leading to negative effects in the biodiversity at recipient sites (Ricciardi and Simberloff, 2009). However, this risk can be minimized with translocations occurring within the same continent that lack major biogeographic barriers whereby invasive properties are unlikely and by using species with low migration rates, such as plants (Mueller and Hellmann, 2008). Despite these concerns, numerous trials are already occurring, such as those for *Torreya taxifolia* that currently occur in northern Florida, but are being translocated to North Carolina (www.torreyagardians.org). However, there are still few assisted migration trials for the purposes of conservation (Hewitt et al., 2011) with more research on this topic needed to improve methods and to gain insight into the challenges and successful approaches associated with this strategy.

I examined assisted migration for *Houstonia longifolia*, a rare and range-restricted herbaceous plant species in Alberta, considered highly habitat specific and dispersal limited (see Chapter 2). The main objectives of this study were to: 1) determine if this species has climate change vulnerabilities; and 2) determine whether translocations to higher latitudes would be a successful conservation strategy to prevent the future decline of this species. We hypothesised that exposure to warmer conditions at southern translocation sites, representing approximately ~2080 climates for the current range, would lead to lower measures of plant performance (survival rates, growth, flower production and seed germination rates) when compared to those in their current range; and 2) translocations to higher latitudes would lead to measures of performance lower than those in the species' current range, but greater than those at the southern translocations.

3.2 Methods

3.2.1 Study species

The long-leaved bluets (*Houstonia longifolia* Gaertner, Moss and Packer, 1983) is a perennial forb in the madder family (Rubiaceae). This species has multiple (1 to 100) short (~15 cm) reproductive stems, opposite leaves, and purplish to white funnel-shaped flowers (Beliveau and Wyatt, 1999; Royer and Dickinson, 2007). It is a distylous species having two distinct floral forms (pins – long-style, thrum – short-style) with a strong self-incompatibility system (Beliveau and Wyatt, 1999). *Houstonia longifolia* is found in five Canadian provinces (Alberta, Saskatchewan, Manitoba, Ontario, and Quebec) and Midwestern and Eastern USA, having a ‘secure’ to ‘apparently secure’ global conservation status of G4G5. Within Canada this species’ status ranges from ‘secure’ and ‘apparently secure’ within Saskatchewan (S4S5), Manitoba (S4S5) and Ontario (S4), to ‘critically imperiled’ within Quebec (S1); and has a conservation status of ‘imperiled’ in Alberta (S2) (NatureServe, 2013). Within Alberta, *Houstonia longifolia’s* distribution is restricted to the Central Parkland Natural Subregion (Alberta Conservation Information Management System [ACIMS], 2015) where it is found on sandy woodlands and dunes (Royer and Dickenson, 2007), or along sandy roadside verge habitat. *Houstonia longifolia* flowers from early June through July (Beliveau and Wyatt, 1999) with seeds maturing by September. This species’ seeds lack any features designed to improve dispersal, with only gravity dispersal occurring from seeds falling out of split ovoid capsules at the top of the stem (Kershaw et al., 2001). Based on its limited dispersal, current level of habitat fragmentation within its range, and the expected rate of climate change, it has recently been ranked the 10th most vulnerable plant to climate change out of 419 rare plant species assessed for Alberta (Barber et al., ‘in press’). Therefore, *Houstonia longifolia* is an ideal candidate for assisted migration.

3.2.2 Translocation sites

In the summer of 2014 native pasture (privately owned) containing a large population (~50,000) of *Houstonia longifolia* was discovered with the help of the Alberta Native Plant Council. This location is referred herein as Gibbons Field, due to its close proximity to the town of Gibbons, AB. This field was to be converted into a highway rest area by 2015 (although negotiations are still in progress as of August 2015). Due to the proposed future destruction of Gibbons Field, permission was granted for the removal of 130 *Houstonia longifolia* plants. In June 2014 plants were collected - along with their source soil to prevent damage to their fine fibrous roots - and placed in half gallon plastic pots. Collections were split evenly between pin and thrum floral morphs. Once collected, plants were given unique ID's containing their floral morph type and then stored outdoors where they were watered until being translocated to experimental sites in the fall of 2014.

In September 2014, 13 *Houstonia longifolia* translocation sites were established at four geographic locations (south, central, north, and far north) across Alberta (Figure 3-1). Each location contained three replicate sites, except for the central location which included four. The south sites were established ~450 km south of this species' current range in Duchess, Alberta. This location was established to determine this species' vulnerability to future climate change, as this area represented the climate as it is expected to be in the 2080s within the species' current range (Schneider, 2013). Four sites were also established within this species' current range (i.e. central location) to act as controls: one site in Gibbons field, one site in Woodbend Forest near the town of Devon, and two sites in the North Bruderheim Provincial Recreation Area. For the northern locations three sites were placed in the Lac La Biche area (about 200 km north of this species' current range) and three sites were placed ~110 km north of Fort McMurray (about 500 km north of species' current range) to test the success of assisted migrations for *Houstonia longifolia* (Table 3-1, Figure 3-1). All sites selected contained sandy soils with open canopy cover.

At each site 10 adult *Houstonia longifolia* plants in half gallon plastic pots were translocated to each site, with an even proportion of pins (five) and thrums (five). Even proportion of pins and thrums were used since this species is believed to have a strong heteromorphic incompatibility system, which requires intermorph crosses (pin x thrum) for seed production (Beliveau and Wyatt, 1999; see Chapter 2). Chicken wire enclosures were used around each pot to prevent herbivory by ungulates (Figure 3-2).

Experimental seed plots were also established near adult *Houstonia longifolia* plants. Seed plots were established at two sites for each location (south, central, north, far north); each consisting of 12 quadrats (~30 cm x 30 cm) separated by a 20 cm buffer. Seed plots at remaining sites consisted of six quadrats, due to limitations in available seed. Seed from pins and thrums was planted in each plot to examine differences in establishment rates between morph types. Since *Houstonia longifolia* does not require wet stratification for germination (only light is needed to break dormancy) (Baskin and Baskin, 1998), seeds were planted in the spring (May) of 2015. Within each seed plot, sand was moistened with water and then seed was dispersed evenly in each quadrat and covered with a thin layer of moistened sand to prevent loss of these small seeds from wind. The number of seeds sown per quadrat ranged from 8 to 2,817.

Average monthly temperature and precipitation data were collected for each location from Environment Canada, along with 30 year normals (1981-2010) for comparisons (Environment Canada, 2015a, 2015b). Weather stations for each location included Fort McMurray, Lac La Biche, Woodbend, and Brooks (near Duchess, Alberta) representing the far north, north, central, and south sites, respectively. For 30 year normals, weather station data were used from Fort McMurray, Woodbend and Brooks (only precipitation normals). However, due to a lack of climate normals for the Lac La Biche and Brooks (missing temperature normals) weather stations, normals were used from weather stations in Athabasca and Vulcan.

3.3 Analysis

In the summer of 2015, all 13 sites were visited and multiple measures of plant performance were recorded. Survival rates were recorded for each plant, with those plants which turned brown being considered dead. Of those plants that did survive, stems were counted and average height – based on up to 10 stems per plant – was measured. From July 2nd to July 4th flowers were counted for each plant. Those flower buds which had yet to open were also included in total flower counts. Seed plots were monitored throughout the course of the summer to measure seedling establishment. Due to the low number of surviving plants and seedling establishment, no statistical analyses were conducted with only summary statistics presented here.

3.4 Results

3.4.1 Adult plants

Eight out of 130 translocated adult plants survived, with no plants surviving at the south and far north locations, and only seven surviving in the central location (17.5%, SE=4.8) and one in the north location (3.3%, SE=3.3) (Figure 3-3). Out of the eight plants that survived, five were thrums and three were pins. The one plant that survived in the north had 22 stems with a maximum height of 6.5 cm and 52 flowers. For the seven plants than grew in the central location, average number of stems was 15 (SE=3), with an average maximum height of 4.7cm, and an average number of flowers per plant of 23 (SE=5) (Table 3-2; Appendix 3-1 to 3-2). The growing season for this species was dry with temperatures being slightly above normal and most sites experiencing less than 50% of the historical precipitation norms from April to May (Appendix 3-3 to 3-4).

3.4.2 Seed plots

No seedling establishment was observed in 2015 despite laboratory germination trials that found viable seed with pin morph seed having a germination rate of 46.7% and thrum morph seed a rate of 32.3% (See chapter 2).

3.5 Discussion

Due to the low survival rate of *Houstonia longifolia* translocations, including those in its current range, it is difficult to assess if this species has climate change vulnerabilities, or whether assisted migration to higher latitudes would improve the success of this species. However, the fact that no *Houstonia longifolia* survived at the south and far north locations suggests that this species is sensitive to climatic conditions. With its low dispersal abilities, *Houstonia longifolia* may not be able to migrate at a pace necessary to keep up with its shifting climatic range. Indeed, it is ranked as the 10th most vulnerable species to climate change based on an assessment of rare plant vulnerabilities to climate change and migration in Alberta by Barber et al. (in press).

The low survival and seedling establishment levels of this species may have been due to the growing conditions in 2015. During this species' growing season (data only available from April to June 2015) temperatures were marginally above normal in April, June and July, but close to normal in May (Appendix 3-3). However, precipitation levels from May to June were less than 50% of the historical norms except for the north site in April, which experienced a slightly higher than normal precipitation level (121.8%) (Appendix 3-4). Precipitation levels at these sites increased in July, but were still below normal precipitation levels, except for the far north location (123.5%). Regardless, the overall growing conditions in 2015 were considered very dry and likely to have contributed to the low survival of the *Houstonia longifolia* transplanted individuals and seed.

At all of the translocation sites both seed and adult plants were used. *Houstonia longifolia* seed plots experienced no germination. This may have been due to seeds being planted in May rather than earlier in the spring during an unusually dry spring and early summer. Translocated adult plants planted in the fall of 2014 fared slightly better than seed, although translocation success was still poor overall. Again this most likely resulted from a lack of moisture during their growing season. However, the root system of this species may have contributed to the low success of these trials with its fibrous roots being too sensitive and frail for translocations. Due to the lack of adult survival and seed establishment for *Houstonia longifolia*, future translocations of this species may require the addition of adult plants (plugs) or amendments to seeding, such as additional watering, shade tents, and the use of seed pucks.

Additional monitoring and trials are needed to gain a better understanding of this species' vulnerabilities and assisted migration potential. However, this lack of successful establishment emphasizes that translocations need to be evaluated on a species-by-species and year-by-year basis, as some species may be more suited for assisted migrations than others (Hanna, 2008; Renton et al., 2012).

3.6 Tables

Table 3-1: Summary of the 13 *Houstonia longifolia* translocation sites at the four locations in Alberta (South, Central, North, and Far North). Total planted per site refers to total number of adult *Houstonia longifolia* plants at each of the 4 locations.

Translocation site name	Location	Latitude	Longitude	Total planted per site	Years since translocated	Total planted
Duchess 2012 Site	South	50.864	-111.886	10	1	30
Duchess Site 1	South	50.903	-112.001	10	1	
Duchess Site 2	South	50.896	-111.884	10	1	
Bruderheim Site 1	Central	53.858	-112.930	10	1	40
Bruderheim Site 2	Central	53.857	-112.929	10	1	
Woodbend/Devon 2012 Site	Central	53.391	-113.761	10	1	
Gibbons Field	Central	53.863	-113.316	10	1	
Lac La Biche Site 2012	North	54.971	-112.001	10	1	30
Lac La Biche Site 1	North	54.982	-112.010	10	1	
Lac La Biche Site 2	North	54.984	-112.009	10	1	
Fort McMurray Site 1	Far North	57.541	-111.337	10	1	30
Fort McMurray Site 2	Far North	57.541	-111.356	10	1	
Fort McMurray Site 3	Far North	57.541	-111.323	10	1	

Table 3-2: Summary of survival, average number of stems per plant, number of flowers, and mean and maximum height for the seven mature translocated *Houstonia longifolia* plants that survived at the Central location and the one mature translocated plant that survived at the North location.

Variable	Central	North*
Total Survival	7	1
Average Number Stems	15 (SE=4)	22
Average Number Flowers	23 (SE=5)	52
Average Mean Height (cm)	3 (SE=0.2)	5.8
Average Max Height (cm)	4 (SE=0.4)	6.5

3.7 Figures

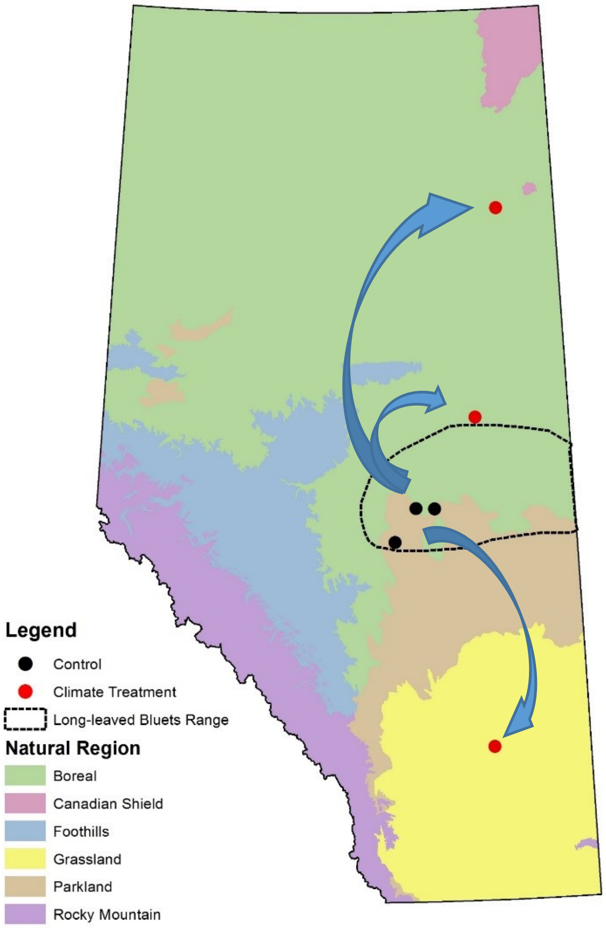


Figure 3-1: Map of Alberta indicating the geographical locations of *Houstonia longifolia* translocation sites (established 2014-2015). Locations north of this species' range were established to test the success of assisted migrations to higher latitudes. The south location was established to test this species' climate change vulnerabilities, as this area is predicted to be similar to the predicted future climate in this species' current range by the 2080s. All plots contained seed and adult *Houstonia longifolia.* Base map from Natural Regions Committee (2006), with diagrams made with assistance from Amy Nixon (ABMI).

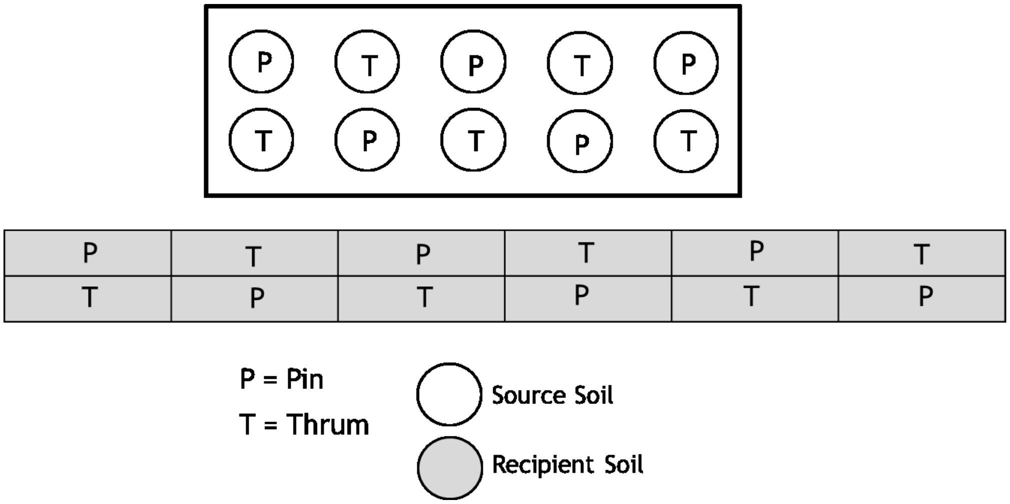


Figure 3-2: Experimental design for *Houstonia longifolia* translocations established in 2014-2015. Ten *Houstonia longifolia* adult plants were placed at each site, five of each floral morph Pin (P) and Thrum (T). All plants were collected from Gibbons Field (Gibbons, Alberta) and placed in half-gallon pots containing source soil from that location. Seed plots contained seed collected from both pin and thrum plants from the Gibbons Field population. Each quadrat (rectangle boxed in diagram) contained seed from one plant, although due to a limitation of seed, seed from some plants was divided between two quadrats. The number of seeds sown per quadrat varied (from 8 to 2817).

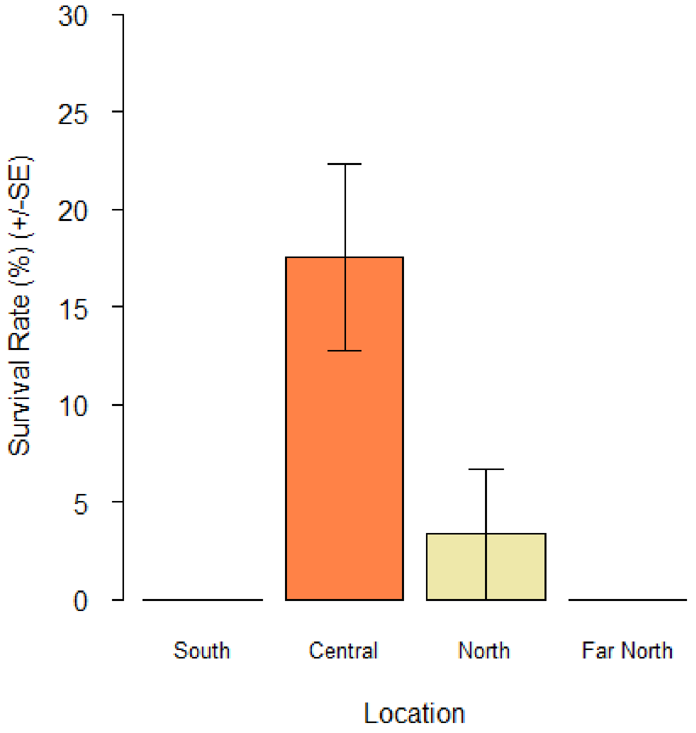


Figure 3-3: Survival (%) of translocated mature *Houstonia longifolia* plants based on those plants surviving at the end of July 2015 from transplanting in the fall of 2014 to four locations in Alberta. Due to the low number of plants surviving, no statistical analysis was conducted on survival. Note that the Central location showed the highest survival rate and represents the species current range.

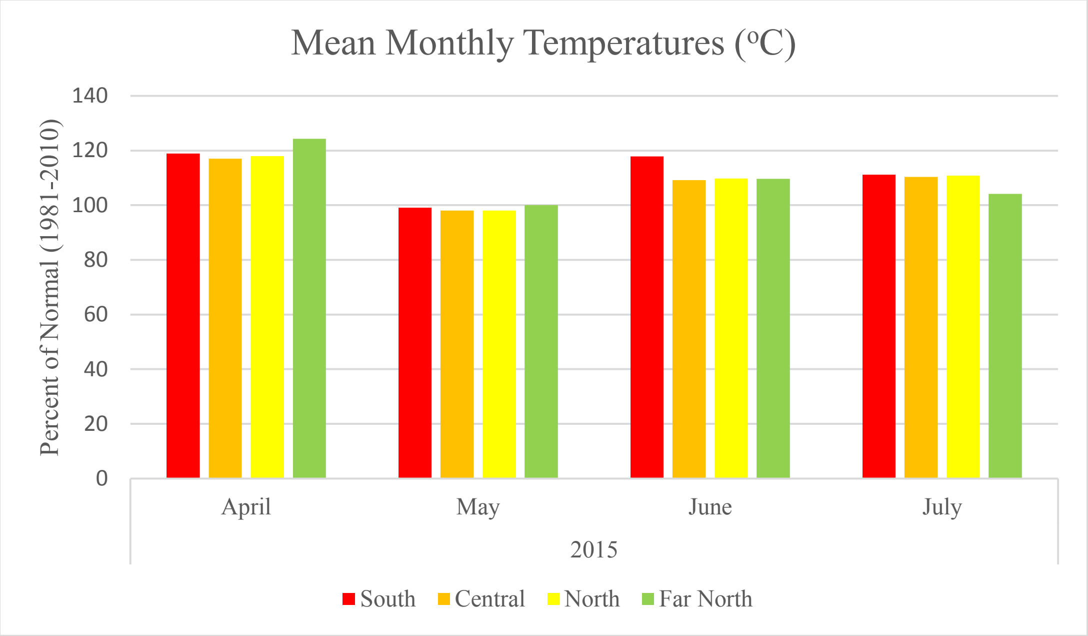
3.8 Appendices

Appendix 3-1: Summary of survival rates (%) (fall of 2014 to July 31 2015) for *Houstonia longifolia* plants across all 13 translocation sites.

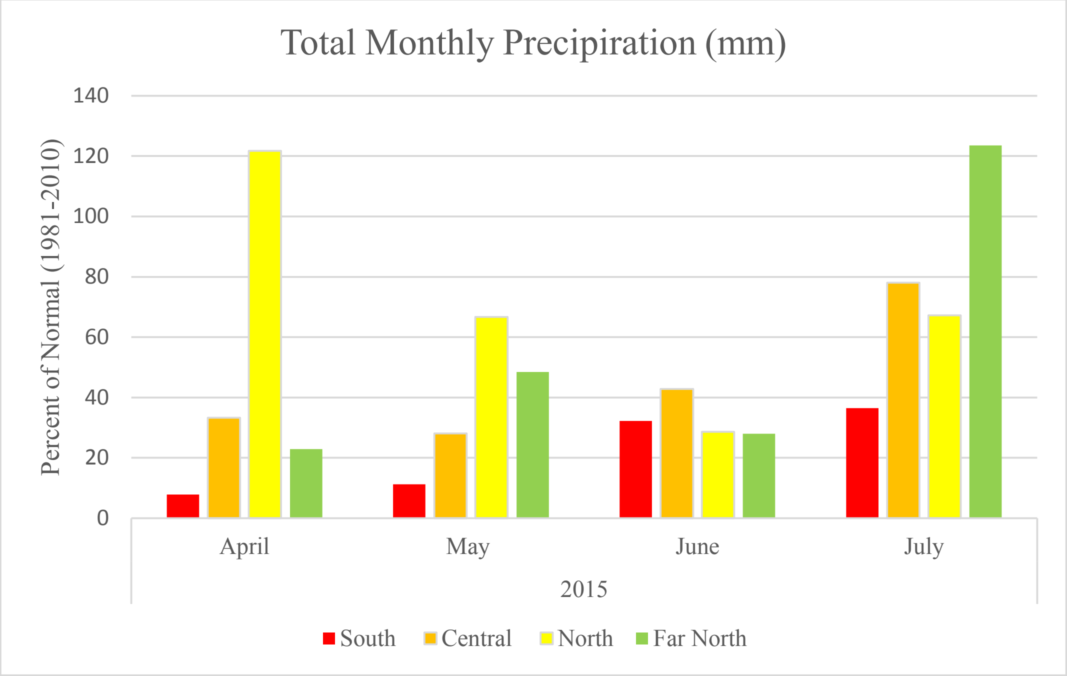
Sites	Location	Survival Rate (%)
Duchess 2012	South	0
Duchess Site 1	South	0
Duchess Site 2	South	0
Gibbons	Central	10
Devon/Woodbend	Central	10
Bruderheim Site 1	Central	30
Bruderheim Site 2	Central	20
Lac La Biche 2012	North	0
Lac La Biche Site 1	North	0
Lac La Biche Site 2	North	10
Fort Mac Site 1	Far North	0
Fort Mac Site 2	Far North	0
Fort Mac Site 3	Far North	0

Appendix 3-2: Summary of attributes of the eight *Houstonia longifolia* plants that survived in 2015 after translocations.

Site	Location	ID	Morph	Stems	Flowers	Mean Height (cm)	Max Height (cm)
Gibbons	Central	15P	Pin	10	5	3.5	6.4
Devon	Central	59T	Thrum	33	34	4.4	6.1
Burderheim Site 1	Central	87T	Thrum	11	17	3.7	5.0
Bruderheim Site 1	Central	106P	Pin	6	21	2.6	3.1
Bruderheim Site 1	Central	109T	Thrum	23	35	3.0	4.1
Bruderheim Site 2	Central	112P	Pin	19	40	3.5	4.3
Bruderheim Site 2	Central	22T	Thrum	5	11	3.1	4.2
Lac La Biche Site 2	North	124T	Thrum	22	52	5.8	6.5



Appendix 3-3: Average monthly temperatures during late spring and early summer of 2015 as a percent of normal (1981-2010) at the four translocation locations based on data from Environment Canada. Monthly temperature data for 2015 were from weather stations in Fort McMurray, Lac La Biche, Woodbend and Brooks for the far north, north, central and south sites, respectively. For the 30 year normals, weather stations in Fort McMurray and Woodbend were used. However due to a lack of temperature normals for Lac La Biche and Brooks, weather stations in Athabasca and Vulcan were used for the north and south sites, respectively.



Appendix 3-4: Total monthly precipitation during late spring and early summer 2015 as a percent of normal (1981-2010) at the four translocation locations based on data from Environment Canada. Monthly total precipitation data for 2015 were from weather stations in Fort McMurray, Lac La Biche, Woodbend and Brooks for the far north, north, central and south sites, respectively. For the 30 year normals, the Fort McMurray, Woodbend and Brooks weather stations were used. However, due to a lack of precipitation normals data for Lac La Biche the Athabasca weather station was used for the north location.

Chapter 4: Experimental use of assisted migration to mitigate the effect of climate change for a rare and range-restricted plant in Alberta: *Liatris ligulistylis*

4.1 Introduction

Species face numerous threats as human development decreases suitable habitat and connectivity (Hannah, 2008; Hewitt et al., 2011). For decades conservation strategies such as protected areas, stepping stones and corridors have been used to promote the persistence of species at risk (Hannah, 2008; Hobbs et al., 2011; Gillson et al., 2013). However, with the increasing pace of climate change and greater fragmentation of habitats, traditional management strategies may not be sufficient to prevent the loss of biodiversity (Hannah, 2008; Hoegh-Guldberg et al., 2008; Schwartz et al., 2012). Additional conservation tactics may therefore be necessary.

Climate change has been linked to shifts in geographical ranges, declines in species distributions, and increases in extinction rates (Thomas et al., 2004; Parmesan 2006; Mahlstein et al., 2013; Dickinson et al., 2014; Lopez, 2015). Based on current climate change scenarios, it is believed that these effects will become even more pronounced (Hamann and Wang, 2006; Parmesan, 2006; Intergovernmental Panel on Climate Change [IPCC], 2007), leading to further declines for species unable keep pace with their shifting climatic niches (Atkins and Travin, 2010; Vitt et al., 2010; Early and Sax, 2011; Ste-Marie et al., 2011; Chauvenet, et al., 2012; Schwartz et al., 2012). Species having low dispersal ability, small population size, long generation times, low adaptation abilities, and that occur within areas lacking connectivity will be at greatest risk (Hunter, 2007; Early and Sax, 2011; Loss et al., 2011; Ste-Marie et al., 2011;

Gallagher et al., 2015). To address these factors, assisted migration has been proposed as a proactive conservation tool (McLauchlin et al., 2007; Thomas, 2011; Schwartz et al., 2012; Renton et al., 2012).

Assisted migration, also referred to as managed relocation (Richardson et al., 2009; Schwartz et al. 2012) or assisted colonization (Hoegh-Guldberg et al., 2008; Seddon, 2010), involves moving organisms to ranges that are predicted to be more suitable under future climatic change scenarios (McLachlan et al., 2007; Loss et al., 2011). This movement can help combat multiple stresses from climate change while overcoming barriers resulting from habitat fragmentation (Renton et al., 2012). However, much debate currently surrounds the use of this proactive conservation tool. This discussion revolves mostly around the risks associated with this strategy, such as the risk of: 1) not taking action, which may lead to extinctions; 2) translocations not being successful; and 3) translocations becoming too successful (Mueller and Hellmann, 2008). The last risk is perhaps the main argument against the use of assisted migrations. By moving a species outside of its range, it may become too successful (i.e. invasive), and thus cause negative impacts on other native species and ecosystem processes (Ricciardi and Simberloff, 2009; Aubin et al., 2011). However, with proper planning and movements within the same continent/biogeographic region and to areas that "lack local endemics" the chances of locally threatened species becoming invasive elsewhere are relatively low (Mueller and Hellmann, 2008; Aubin et al., 2011; Hewitt et al., 2011; Thomas, 2011). In order to deal with the multiple social, political, economic and ecological issues, several decision-making frameworks that assess the use of this conservation tool have been proposed (Hoegh-Guldberg et al., 2008; Richardson et al., 2009; McDonald-Madden et al., 2011; Schwartz et al., 2012).

Although there is still no fully accepted framework for its use, numerous assisted migrations are currently underway. For instance, trials have occurred for *Torreya taxifolia* (www.torreyagardians.org), *Pinus albicaulis* (Whitebark pine; McLane and Aitken, 2012), and two butterfly species in the U.K. (Willis et al., 2009). Assisted migration trials are also being used by the forestry sector in Western North America (e.g. Assisted Migration Adaption Trial Project) to assess the performance of 16 tree species after translocation to northern latitudes (Marris, 2009; Williams and Dumroese, 2013). These trials are important as they provide ecological evidence necessary for use in decision making frameworks. They also provide an assessment of methods and protocols for future translocations, although admittedly the emphasis of tree translocations is often less around conservation and more around economic forces (yield), and mostly focused on common species that are not threatened (Aubin et al., 2011). The translocation of species has a long-history, whether it be the movement of plants for human needs (e.g., aboriginal values, agriculture and horticulture), the revegetation of disturbed areas by restoration ecologists, the study of populations and adaptation by scientists using common garden or provenance trials, or the reintroduction of extirpated species by conservation biologists (Mack and Lonsdale, 2001; Vitt et al., 2010). However, as outlined by Hewitt et al. (2011), the number of studies of assisted migration trials that focused on mitigating the impacts of climate change are still uncommon in scholarly literature. This study will provide additional insight into the success and challenges of using assisted migrations by examining translocation trials for a locally rare herbaceous plant species – the northern blazing star (*Liatris ligulistylis*). Plants are ideal candidates for these trails since they are among the most limited in dispersal ability (Field, 2014), and have a lower chance of invasiveness compared to fish, crustaceans and mammals (Mueller and Hellmann, 2008).

Specifically, my objectives for this study were to: 1) evaluate vulnerabilities to projected climate change (i.e. future warming) through translocations south of this species' current range; and 2) determine the potential use of assisted migration as a conservation tool for this species, through translocations to higher latitudes outside of its current range. These objectives relate to two related hypotheses: (1) exposure to warmer conditions at southern translocation sites representing approximately ~2080s climates for the current range will lead to lower survival rates, growth (height), reproduction (flower buds, and seed production), and colonization (seedling emergence/establishment levels), as compared to the species' current range; and 2) northern translocations outside of this species' current range will lead to lower plant performance (survival, growth, reproduction and germination) than those in the species' current range, but greater measures of performance than those at the southern translocation sites. Support of the first hypothesis would suggest near future (2080s) vulnerabilities to warming, while the second would support that the current range represents the most suitable climatic conditions representing climatic equilibrium conditions and thus a narrow range of conditions suitable for the species. If plant performance in the north is similar or exceeds that of the current range, then the current distribution of the species would be limited by historic dispersal barriers that would support hypotheses of migration lags and disequilibrium conditions.

4.2 Methods

4.2.1 Study species

The northern blazing star (*Liatris ligulistylis* (A.Nels.) K.Schum., Moss and Packer, 1983) is a perennial forb in the family Asteraceae. It has stem heights between 30-80 cm, alternating leaves, and multiple purple heads with disc florets (Royer and Dickinson, 2007). The root system contains corms, which are useful for translocation of adult plants during dormancy. It has a global conservation status of 'secure' (G5), and is found in Canada (Alberta, Saskatchewan and Manitoba) and the Midwestern USA. Conservation status in the USA ranges between S1 and S3 for those states in which it has been assessed (Colorado: S1S2, Iowa: S3, Nebraska: S1, Wyoming: S2), while presumably secure in the other seven states which have yet to be assessed (Connecticut, Illinois, Minnesota, Montana, New Mexico, North Dakota, South Dakota, and Wisconsin). For Canada, *Liatris ligulistylis* has a conservation status of 'secure' (S5) in Saskatchewan, and 'apparently secure' (S4) for Manitoba, with a status of 'vulnerable' (S3) in Alberta (NatureServe, 2013). In Alberta the range of the species is restricted to the Central Parkland Natural Subregion representing the northern and western most distribution of this species (Alberta Conservation Information Management System [ACIMS], 2015). Due to its limited distribution in Alberta's Central Parkland, it is believed to have a narrow climate niche, as no occurrences are found to the north (cooler boreal forest) or to the south (drier and warmer grasslands). A related species, *Liatris punctata,* is found to the south, but not to the north. *Liatris ligulistylis* is usually found on open woodlands, moist meadows, and sandhills (Royer and Dickinson, 2007), with extant populations in Alberta restricted to stable isolated Aeolian sand dunes, and sandy verge habitat such as roadsides.

It blooms in late July to mid-August, and from September to November its seeds are dispersed by wind with the help of a bristly pappus attached to the achene (Royer and Dickinson, 2007). Although data are lacking for this species' dispersal abilities, as is the case for many plant species (Royal Botanic Gardens Kew, 2015), a status report for *Liatris spicata*, in the same genus with similar seed morphology, indicated only moderate dispersal success over short distances (COSEWIC, 2012). Therefore, *Liatris ligulistylis* is believed to have a limited long-distance dispersal ability; this would limit it from migrating with its shifting climatic range, especially given the high levels of habitat fragmentation that exist within this species' current range of the Central Parkland (Barber et al, 'in press'). Based on the above characteristics, *Liatris ligulistylis* is considered to be an ideal candidate for assisted migration trials.

4.2.2 Population surveys

In the summer of 2013 line transects were used to locate *Liatris ligulistylis* populations in six different provincial protected areas in central Alberta: Northwest of Bruderheim Natural Area (hereafter Northwest Bruderheim), North Bruderheim Provincial Recreation Area (hereafter North Bruderheim), Bellis Lake Natural Area (hereafter Bellis Lake), Halfmoon Lake Natural Area, Redwater River Natural Area, and Opal Natural Area. Line transect surveys involved walking parallel 500 m transects between two pre-determined GPS points and recording the location of all *Liatris ligulistylis* individuals (Appendix 4-1). These population surveys were conducted to better understand this species' habitat and to locate populations large enough that individual plants could be removed for assisted migration trials without affecting their long-term population viability.

Occurrences of *Liatris ligulistylis* were found in three of the six surveyed areas: Northwest of Bruderheim, Bellis Lake and North Bruderheim. Only small numbers of plants were found in Northwest Bruderheim and Bellis Lake with large numbers of individuals found in North Bruderheim. To specifically quantify population size for the larger population in North Burderheim, additional line transect quadrat surveys were conducted. These surveys consisted of 61 parallel 100 m transects (270° bearing) with 1-m^2 circular quadrats systematically spaced every 5 m (n=1215). In each quadrat the density of individual plants were counted.

In addition to density, measurements recorded when the species was present included: species composition (% cover for all vascular plant species), canopy cover (% cover over one plant), bare ground (%), slope, aspect, and average height and number of florets. Average plant density was estimated at 0.22 plants/m^2 (SE=0.05). With total amount of suitable habitat in North Bruderheim estimated at ~40 ha, total population size of *Liatris ligulistylis* was estimated to be ~88,000 (+/- 8,624).

4.2.3 Translocation experiment

Three *Liatris ligulistylis* translocation sites were established across Alberta in October of 2012, one in the south (Duchess), one in this species' current range (Woodbend Forest), and one in the north (Lac La Biche). At each site 10 *Liatris ligulistylis* corms from mature plants were translocated from populations collected in September 2012 along roadside verge habitats in the Ukalta and Bruderheim areas (Figure 4-1, Figure 4-2). During collection corm weight, width and height of each plant were recorded. Seed plots consisted of 200 seeds planted at each site at the time of corm translocation. However, these plots had little or no seedling establishment in addition to the destruction of the southern site from third party activities. Therefore, a sowing trial was established in 2013 in which 1000 seeds, collected from plants from the largest extant population at North Bruderheim, were sown at each of these three existing 2012 translocation sites (Figure 4-1).

After surveys in the summer of 2013 found North Bruderheim to contain a large population of *Liatris ligulistylis,* permission was granted from Alberta Tourism, Parks and Recreation (Permit No: 13-132) to remove 150 plants from the area. Plants were removed in late August from different areas of the site and in numbers so as to prevent declines in any single sub-population following recommendation from Lancaster (2000). The floral stem was removed from each plant and placed in a paper bag marked with its GPS location. The corm was placed in a plastic bag containing soil from where each plant was removed and labeled with a matching GPS location. In the lab at the University of Alberta, paper bags containing the floral stems were placed in windowsills for seed drying. Plastic bags containing corms with source soil were placed in a growth chamber at 10 degrees Celsius until they were ready for translocation.

In October 2013, nine additional translocation sites were established using 144 of the 150 corms collected as well as corresponding seed removed from North Bruderheim. At each of the nine new translocation sites, 16 adult corms were planted and 1000 seeds were sown. At each site, 10 seed plots (~30 cm x 30 cm) were delineated and separated by a 20 cm buffer. The soil was lightly raked in each of the 10 plots with 100 seeds sown in the middle and thinly covered with soil (Figure 4-1, Figure 4-3).

At both the 2012 and 2013 translocation sites the corms were placed into 2 gallon plastic pots with recipient or source soil randomly assigned to each pot. This method allowed for the separation of climatic and edaphic factors, although sites for translocation were targeted to be similar (sandy soils). It should be noted that the sites in North Bruderheim all contained the same soil (source) as they were placed in the same area from which they were taken. Enclosures made from chicken wire were established around each pot (Figure 4-2, 4-3) to prevent herbivory of adult plants, as deer grazing on *Liatris ligulistylis* was observed.

From 2012 to 2013, a total of 12 *Liatris ligulistylis* translocation sites were established, consisting of both adult corms and seed. Three of these experimental sites were in southern Alberta near Duchess, about 450 km south of the species' current range thus assessing vulnerability to future climate change, as this area is expected to reflect the future 2080s climate within its current range (Schneider, 2013). Six experimental sites were established in northern Alberta, north of the species' current range, with three sites in the Lac La Biche area (~200 km north of this species' current range), and three sites north of Fort McMurray (~500 km north of this species' current range). Finally, three sites were established within this species' current climatic range; two in North Bruderheim and one in Woodbend Forest near the town of Devon. These three sites act as controls as they are within this species' currently occupied climatic range, although the Woodbend site does not have extant populations or past records of this species, but similar soils. The twelve sites were evenly divided among the four locations: south, central, north and far north, each containing three replicate sites (Figure 4-4, Table 4-1).

In the spring of 2014, timelapse cameras were established at all 12 *Liatris ligulistylis* sites, and programmed for four pictures per day between 11:30 am to 1:00 pm. These pictures captured phenology of the adult *Liatris ligulistylis* plants including first sprouting date and bud burst for the 2014 growing season. In the summer of 2015, composite soil samples were collected from each site and analyzed to determine variations between source and recipient soils. The Dumas combustion method was used to determine total nitrogen (TN) and total organic carbon (TOC) of these soil samples. Then electrical conductivity (EC) and pH were recorded with the use of a pH/EC meter. Finally, to determine phosphorus and potassium levels a Modified Kelowna extraction occurred, followed by an Atomic Absorption analysis for potassium and Colorimetric methods for phosphorus (Appendix 4-9).

Average monthly temperature and precipitation data were gathered for each location from Environment Canada weather stations, along with 30 year normals (1981-2010) for comparisons (Environment Canada, 2015a, 2015b). For monthly averages, weather stations used included Fort McMurray, Lac La Biche, Woodbend, and Brooks (close to Duchess, AB) for the far north,

north, central, and south sites, respectively. For 30 year normals, weather stations data were used from Fort McMurray, Woodbend and Brooks (only precipitation). Lac La Biche and Brooks lacked long-term temperature normals. Normals were used from nearby weather stations in Athabasca and Vulcan. Mean annual temperatures (MAT) and mean annual precipitations (MAP) were gathered for each location, along with average growing season temperatures and precipitation levels from May to August 2014. These growing season conditions were then compared with 30 year normals.

4.2.4 Response measures

In 2014, a number of measures were recorded at each of the 12 *Liatris ligulistylis* translocation sites for adult plants. Survival rates and the proportion of plants that bloomed were recorded for each site by noting the number of plants that produced aboveground leaves, and of those that survived how many contained flowering stems. Stem heights were measured to the tip of the stem for those non-flowering stems, and to the base of the flower bud at the top of the stem for those with flowering stems. Due to this species having numerous stems, average plant height was calculated by summing the heights of all stems of a given plant and dividing by the total number of stems. The height of the tallest stem from each plant was recorded as the plant's maximum height. Number of flowering buds per plant was also recorded by summing the number of buds on each flowering stem.

To quantify seed production mesh begs were placed around flowering heads at the start of senescence to prevent loss of seeds. Those stems contained in these mesh bags were cut just below the tie of the bag, and were taken to a lab at the University of Alberta for further drying and counting of seed. During collection two plants had their seed bags ripped by the chicken wire enclosures, and therefore were removed from analysis. Recounts of a 5% sub-sample of these counts were used to assess quality control and found an average error rate of 0.27% (Appendix 4-12). During seed counting the proportion of full seeds was determined by lightly pressing seeds between fingers to assign whether seeds were ‘full’ or ‘empty’. With ‘empty’ referring to those seeds which lacked an embryo.

Measures of plant performance were also considered for seed emergence during the 2014 growing season. Those seeds which germinated and emerged as seedlings between May-August 2014 were recorded and referred to as seedling emergence. Seedlings that were still alive at the end of August 2014 were also recorded and referred to as seedling establishment. Average seedling height was also calculated by measuring up to 10 seedlings in each quadrat.

4.3 Analysis

The statistical software package R. 3.1.2 was used in all analyses (R Core Team, 2014). The interaction term between location and soil was initially included in each model, but removed when non-significant. All model residuals were checked for normality and homoscedasticity with outliers removed. The lsmeans package (Lenth and Hervé, 2015) was used to calculate means for all measures of plant performance, and for post-hoc comparisons using a tukey adjustment. An alpha value of 0.05 was used for removal of interaction terms, and the significance of location, soil and for post-hoc comparisons.

4.3.1 Adult plants

For the analysis of survival those plants that did not sprout aboveground leaves were considered mortalities and assigned a value of 0, while those that did grow were given a value of 1. A similar approach was used for the proportion of plants that bloomed, with those plants that did not produce a flowering stem being assigned a value of 0, and those that did a value of 1. For these variables generalized linear models with a binomial distribution - using the package nlme (Pinherio et al., 2015) - were used to determine the effect of soil and location, with site as a random effect. Due to the lack of variation explained by the random effect, it was removed from final analyses. Likewise, the interaction between location and soil type was not significant ($p<0.05$) and was therefore removed from all models. A likelihood ratio test was estimated using the drop1 function to determine the significance (χ^2 distribution) of location and soil by single term deletions (Table 4-2).

A linear mixed-effect model using the package nlme (Pinheiro et al., 2015) was used to determine the effect of soil and location on maximum height in the 2014 growing season, with site as a random effect. The interaction between soil and location was non-significant ($p=0.772$) and therefore dropped from the final analysis (Table 4-2). Maximum height was used instead of average height as it is believed to give a more accurate representation of growth, because plants with multiple tall shoots can contain short stems leading to underestimation of height.

Generalized linear mixed-effects models, with a Poisson distribution using the package lme4 (Bates et al., 2014) were estimated to test the effect of location and soil type on the number of flower buds (site as a random effect) and total seed production per plant (plant ID nested within site as a random effect). For both models, the interaction between soil and location was found to be non-significant ($p=0.190$, $p=0.830$) and was therefore removed from the final model. Because the significance of factors is not provided in the lme4 package, a parametric bootstrap set at 4999 iterations was done to assess significance using the afex package (Singmann et al., 2015). However, due to convergence issues a likelihood ratio test, using the afex package (Singmann et al., 2015), was used to determine significance (χ^2 distribution) of location and soil on seed production (Table 4-2).

Proportion of full seeds for each plant was calculated by dividing the number of full seeds by the total number of seeds produced. No individual plant contained all full seed, but there were several which contained none. Proportion of 0.0 full seeds were replaced by 0.001 to allow for an Arcsine transformation of the data. A linear mixed effects model was estimated using the package nlme (Pinheiro et al., 2015) on the transformed proportion of full seeds to determine the effect of location and soils. In this model the random effect was each plant (ID) nested in site.

Due to the spherical shape of the corms, the equation of $V=4/3\pi r^3$ size was used to calculate corm size. Radius (r) in this equation was calculated by averaging width (average based on two measurements taken at right angles to one another) and height and dividing by two as follows: $r = ((\text{Width 1} + \text{Width 2})/2 + \text{Height})/2)/2)$. Linear mixed effects models were used (package nlme; Pinheiro et al., 2015) to determine the effect of corm size on plant height and number of buds in the 2013 and 2014 growing season with site as a random effect. Dates on which the first plant showed first sprouting and first bud burst (when the top (first) bud opened) were estimated from photographs obtained from timelapse cameras.

4.3.2 Seed plots

A negative binomial generalized linear mixed effects model was used (package lme4; Bates et al., 2014) to test the effect of location on seed emergence and establishment since the Poisson model was over-dispersed ($\varphi > 4$). Because only recipient soil was used, location was the only predictor in this analysis, with site as a random effect. Significance values were not provided in the lme4 package, therefore parametric bootstraps set at 4999 iterations were used to obtain significance for these models using the afex package (Singmann et al., 2015). Also a linear mixed effects model from the package nlme (Pinheiro et al., 2015) was used to assess the effect of location on average seedling height, with site as the random effect.

4.4 Results

4.4.1 Summary of surveys for a natural population

Based on summer 2013 quadrat observations for natural populations of *Liatris ligulistylis* in North Bruderheim, 33.6% of *Liatris ligulistylis* were flowering (64.1% were basal, juvenile plants or mature plants failing to flower) and 2.6% were considered to have been browsed. Average number of florets per plant was 11 (SE=1), average flowering plant height was 47.1 cm (SE=4.1), and average basal plant height was 17.2 cm (SE=1.7). Generally in this area, species grew in patches on mid slopes, often with flat or westward aspects. Most of the basal plants that were assumed to be juvenile plants were observed near mature flowering plants, suggesting low dispersal abilities, but also successful recruitment following a fire in 2009. It was also observed that plants were associated with sandy habitats with a ground cover consisting mostly of grasses/sedges and forbs/sub-shrubs thriving in areas of low competition with bare ground present in most of the plots where the species occurred (Appendix 4-2 to 4-4).

4.4.2 Adult plant translocations

Neither location, nor soil type (source or recipient) had a significant effect on survival, which was relatively high overall (south: 73.8%, SE=7.0, central: 86.4%, SE=5.6, north: 87.8%, SE=5.1, far north: 79.2%, SE=5.9) (Table 4-2, Figure 4-5). Location and soil type also did not significantly affect the proportion of plants that bloomed, with most bloom rates being close to 50% (south: 41.5%, SE=8.9, central: 41.5%, SE=8.8, north: 44.9%, SE=8.3, far north: 50.0%, SE=8.1) (Table 4-2, Figure 4-6).

The far north location had the greatest average maximum plant height (37.4 cm, SE=2.4 cm) followed by the north (34.1 cm, SE=2.4), south (28.1 cm, SE=2.6), and central (27.8 cm, SE=2.6) locations (Figure 4-7). The overall effect of location on maximum height was marginally significant ($p=0.068$, Table 4-2), with pairwise comparisons not finding any significant ($p<0.05$) differences between locations (Appendix 4-5). Nevertheless, these results indicate that this species was able to survive and grow to full or greater heights even 500 km outside of its current range. Maximum heights were, however, shorter than the heights of the natural population (average = 47.1 cm, SE=4.1), including central locations within its range, suggesting that disturbance of plants from translocation negatively affected plant growth. Soil type had no significant effect on maximum plant height with those plants in source soil being only marginally taller (34.8 cm, SE=1.67) then those planted in recipient soil (30.1 cm, SE=2.3) (Table 4-2).

Soil type had a significant effect on flower bud production ($p = <0.001$), with those plants in source soil producing nearly 1.5 times as many buds as those placed in recipient soil (Table 4-2, Figure 4-8). However, location had no significant effect on the number of buds, producing on average between 9 and 12 buds per plant across all locations.

The amount of seed produced per plant ranged from 39 to 2865 seeds. Seed production was the greatest at the far north location (423.3, SE=95.2), followed by the central (372.8, SE=82.0), north (246.8, SE=53.1) and south (209.5, SE=48.6) locations (Figure 4-9). However, location was found to have no significant effect. Soil type also had no significant effect on seed production with those plants in source soil only producing slightly more seed (319.1, SE=38.5) than those planted in recipient soil (283.0, SE=39.9) (Table 4-2).

Proportion of full seeds per plant ranged from 0.00 to 0.857 with an average proportion of 0.229. A significant interaction was found between location and soil type (p=0.018). Post hoc pairwise comparisons demonstrated that the proportion of full seeds was higher for recipient soil at the central location than at the south or far north location, while values at the north site were intermediate in proportion full seeds. No significant differences were found in the proportion of full seeds among locations for those plants in source soil. At the south location, source soil produced more full seeds than the recipient soil, although recipient soil in the central and north locations produced marginally more full seeds than those plants placed in source soil (Table 4-2, Figure 4-10, Appendix 4-6 to 4-7).

Corm size had no significant effect on maximum heights of *Liatris ligulistylis* in 2013 or 2014 or number of buds produced in 2013. Corm size did have, however, a significant positive effect on the number of flower buds produced in 2014 (p=0.007; Table 4-3). Since not all corms were used in this analysis, corm size could not be used as a covariate in other analyses. However, these results indicate that, not surprisingly, the size of corms may explain variation in bud and seed production among *Liatris ligulistylis* plants with larger corms resulting in higher reproductive rates. Thus larger corms could be selected preferentially for future assisted migration trials.

Based on timelapse photographs in 2014, the south location sprouted first (May 6th - May 20th), followed by the central (May 11th - May 25th), north (May 19th - June 1st), and far north (May 31st - June 4th) locations. Date of first sprouting for *Liatris ligulistylis* was therefore inversely related to latitude and presumably early spring climate conditions. A similar trend with latitude was observed for first bud burst. First bud burst occurred first at the south location (July

13th – July 21st), followed by the central (July 20th – 24th), north (July 18th – August 1st) and far north (July 28th – August 3rd) locations (Appendix 4-8).

Recipient soil at the south location and Woodbend Site (central location) had higher total organic carbon (TOC), total nitrogen (TN), pH, electrical conductivity (EC), and potassium (K), but lower phosphorus (PO_4-P) than the source soil from North Bruderheim. In contrast, the north and far north recipient soils had lower TOC, TN, EC, and K levels (except Lac La Biche Site 1), but higher PO_4-P concentrations and similar pH values than the source soil. Recipient soils at all locations also had similar textures with silt having the highest percentage particle concentration followed by sand and clay (Appendix 4-9).

Mean annual temperatures (MAT) decreased with latitude with the far north location having a mean annual temperature of 0.0 ^{o}C. This was 2.7 ^{o}C cooler than the central location (i.e. species current range). Mean annual precipitation (MAP) was marginally higher at the north location (416 mm), with the far north location (384 mm) and central location (384 mm) having identical mean annual precipitations. The south location had the lowest mean annual precipitation (330 mm). The 2014 growing season mean temperatures (May - August) were similar across all locations with the south location having the highest average temperature of 15.6 ^{o}C. These growing season temperatures were slightly above the 30 year normals. Growing season precipitation (May – August) increased with latitude with the far north and north locations having the highest precipitation. However, when compared to 30 year normal, all sites experienced growing season precipitation equal to or below their long-term normals (Table 4-4) demonstrating droughty conditions during the 2014 growing season (especially springtime conditions).

4.4.3 Seed translocations

Location had a significant effect on both seedling emergence and seedling establishment (p=<0.001, Table 4-2). Post hoc comparisons illustrated that seedling emergence and establishment rates were significantly different between all locations except the north and far north locations, with the north and far north locations having significantly higher emergence rates than the south and central locations (Figure 4-11, Appendix 4-10 to 4-11). However, location had no significant effect on seedling maximum height, with heights being similar among locations (south: 3.8 cm, SE=0.7, central: 3.6 cm, SE=0.5, north: 4.8 cm, SE=0.5, far north: 4.6, SE=0.5) suggesting that growth once seedlings emerged did not respond to different weather conditions (Table 4-2, Figure 4-12).

4.5 Discussion

This study found that *Liatris ligulistylis* can survive, grow, flower and produce seeds hundreds of kilometers outside of its current range, especially to the north of its current range. These results suggest that this species may be a good candidate for future assisted migrations to mitigate climate change impacts on this rare and range-restricted plant; however, this is a preliminary study and continued monitoring is needed to determine the long-term success of this conservation tool. The inclusion of source soil was also found to improve plant performance of translocated mature plants to higher latitudes.

It was hypothesized that the south locations would experience declines in survival rates, bloom proportions, heights, fecundities and germination levels, indicating climate vulnerabilities. Translocations of adult *Liatris ligulistylis* plants (corms) did not support this hypothesis for most measures, except for proportion of full seeds that was significantly lower at the south location than at the central location. Otherwise there were no significant effects found of location on any of the other measured characteristics for translocated adult plants (i.e., survival rate, proportion that bloomed, maximum height, number of buds, and total seed production). However, seedling emergence and establishment were significantly lower in the south location than at the central location suggesting that this species may have future climate vulnerabilities in recruitment thereby limiting viability of existing populations with climate change.

It was hypothesized that assisted migrations of *Liatris ligulistylis* to northern locations would result in lower levels of survival, growth, reproduction and seedling emergence/establishment than those in the central location (current range). Surprisingly, there were no significant differences in survival rates and maximum heights between the northern and central locations. This species was therefore able to survive well north of its current range, and grow to heights similar or higher than those at the central location (i.e. current range). Also, these northern translocated plants had bloom percentages, bud abundances and total seed production levels similar or higher than those in its current range. The realized climatic niche of this species therefore appears to be a restriction of its broader fundamental niche which includes cooler conditions to the north. Perhaps suitable edaphic sites to the north are too isolated and thus unoccupied, but climatically already suitable.

Seedling emergence/establishment levels in the central locations (i.e. current range) were significantly lower than those at the northern locations. This suggests that *Liatris ligulistylis* is at non-equilibrium conditions with the climate in its current range. This phenomenon has been observed in a study by Sposajevic et al. (2014), where the translocation of seed from two plant species (*Horkelia sericata* and *Phacelia corymbosa*) to cooler microclimates (i.e. higher elevations and northern aspects) led to higher germination rates than those in their current range.

It is likely that numerous species are currently or will soon be out of equilibrium with their current range, especially those herbaceous plant with limited dispersal abilities (Willis, 2009; Atkins & Travin, 2010; Field, 2014).

Inter-annual variation in weather conditions can affect the success of translocations, and may have contributed to the higher seedling emergence/establishment rates observed at northern locations. Monthly precipitation and temperatures compared to climate normals (1981-2010) found that the 2014 average temperatures and precipitation levels at assisted migration sites (north and far north locations) were similar to historic conditions, except for the month of May where the far north location experienced precipitations levels above normal (221.7%) (Appendix 4-13, Appendix 4-14). However, the north and far north sites experienced similar levels of seedling emergence and no significant differences in heights, survival, blooming percentage or total seed produced. Therefore, it is unlikely that the above normal precipitation in May at the far north site had a significant effect on this species' growth. These yearly fluctuations in temperatures and precipitation may, however, dictate the timing of translocations, as well as any amendments necessary for successful establishments (e.g. watering, shade tents).

This study provided insight into the protocols and methods needed for future trials. At all locations, planting of *Liatris ligulistylis* corms in source soil resulted in significantly more flower buds than planting in recipient soils. At the south location the proportion of full seeds was also greater for plants that were translocated with source soil. The recipient soils at the south location contained higher levels of total organic carbon, and nitrogen, which could make it a better choice depending on measures of plant performance, such as plant height. However, this soil also had higher pH and EC values, along with lower phosphorus levels than those in the source soil, which could have contributed to differences in the proportion of full seeds observed among sites.

Although the proportion of full seeds was greater in the source soil at the south location, this measure was marginally greater for those plants placed in recipient soil at the central and north location. This may have been caused by the higher total organic carbon of the recipient soil at the central site; or possibly by the lower EC and higher phosphorus levels of the recipient soil at the north location. However, recipient soils are not likely to be identical to the source soil and therefore their inclusion in assisted migration could negatively affect other measures of growth such as the number of buds which was observed in this study.

Source soils could also contain mycorrhizal fungi, which will promote the growth of species with mycorrhizae associations (Schwartz et al., 2006). *Liatris ligulistylis* is believed to have vesicular-arbuscular mycorrhizal associations, as they have been found for a similar species *Liatris spicata* (Medve, 1985; COSEWIC, 2012). Therefore, the increase in bud production and proportion of full seeds (south location) may be due partly to these associations being maintained through the use of source soil. In fact, it has been suggested that "paired or multispecies" translocations may improve the success of assisted migrations (McLachlan et al., 2007). Therefore, future studies should consider any and all mutualisms for those species being translocated. Management practices for these mutualisms could include, for example, inoculation of mycorrhizae to promote growth (Gallagher et al., 2015; Schwartz et al., 2006) or the movement of pollinators along with those specialist plants which depend on certain pollination mutualisms.

This study has shown that soil can affect the success of these assisted migrations. Therefore, future trials should incorporate source soil along with translocation of plants. However, thorough investigations into these soils should occur to prevent the translocation of invasive flora or fauna. If the use of source soil is not possible then recipient soil at proposed translocation sites should be tested to make sure these recipient soils are as similar as possible to the source location.

To gain insight into whether future assisted migrations should use seed or adult plants, both were used at each translocation site. Although, the translocation of seed is easier, more cost efficient, and less likely to cause declines in current population levels, this study found seedling emergence/establishment rates to be lower (~20%), than the survival rates of adult plants (~80%). However, seed has been used successfully in other translocation studies (McLane and Atiken, 2012; Spasojevic et al., 2014), and also has the unique advantage of being able to be stored in seed banks (e.g. Millennium Seed Bank Project of the Royal Botanic Gardens, Kew) until conditions become ideal at recipient sites (Vitt et al., 2010). These seed banks provide "a safety net from both natural and human uncertainty" by giving time for debate, investigation and the formulation of plans for a species' assisted migration (Vitt et al., 2010). With extinction rates rising, seed from these banks may be the only option available for future assisted migrations of some species. Therefore, current trials should include adult plants along with seed as these plants are believed to improve colonization rates within a location and allow for natural regeneration by the dispersal of their seeds.

With the continuing pace of climate change and level of human disturbance on the landscape we need to move away from the ideal of restoring the landscape back to a pre-distributed natural state (Hobbs et al., 2010; Hobbs et al., 2011; Thomas, 2011; Higgs et al., 2014). By 2050, under mid-range climate warming scenarios, 15% to 37% of species will be "committed to extinction", with higher rates expected for those with dispersal limitation (Thomas et al., 2004). Therefore, new conservation strategies, such as assisted migration need to be tested, as they could be successful in preventing the loss of rare and range-restricted species

(McLachlan et al., 2007). This study has provided insight into the use of assisted migration using a short-term study of *Liatris ligulistylis*. However, in order to determine the true success of this conservation strategy continued monitoring and additional trials are necessary. Although there are risks and uncertainty with this conservation tool, there are also significant benefits such as the preservation of biodiversity for future generations (Aubin et al., 2011).

4.6 Tables

Table 4-1: Summary of the 12 *Liatris ligulistylis* translocation sites at the four locations in Alberta (South, Central, North, and Far North). Total planted refers to total number of adult *Liatris ligulistylis* plants at each of the 4 locations.

Translocation site name	Location	Latitude	Longitude	Total planted per site	Years since translocated	Total planted
Duchess 2012 Site	South	50.864	-111.886	10	2	42
Duchess Site 1	South	50.903	-112.001	16	1	
Duchess Site 2	South	50.896	-111.884	16	1	
Bruderheim Site 1	Central	53.858	-112.930	16	1	42
Bruderheim Site 2	Central	53.857	-112.929	16	1	
Woodbend/Devon 2012 Site	Central	53.391	-113.761	10	2	
Lac La Biche Site 2012	North	54.971	-112.001	10	2	41
Lac La Biche Site 1	North	54.982	-112.010	15*	1	
Lac La Biche Site 2	North	54.984	-112.009	16	1	
Fort McMurray Site 1	Far North	57.541	-111.337	16	1	42
Fort McMurray Site 2	Far North	57.541	-111.356	16	1	
Fort McMurray Site 3	Far North	57.541	-111.323	16	1	

* 16 were planted but one was lost due to vandalism

Table 4-2: Effect of soil, location and their interaction on measures of *Liatris ligulistylis* performance in 2014. The interaction between soil and location was only significant for proportion of full seeds and the interaction term was therefore only retained for that model. Seedling emergence refers to the percent of seeds that germinated and emerged as seedlings in 2014, whereas seedling establishment refers to the percent of seeds that germinated, emerged as seedlings and were still alive at the end of August 2014. Stat refers to parametric bootstrap statistics used to calculate p-values. **Bolded text** indicates values obtained by running a likelihood ratio test (LRT). '*' indicates $p < 0.05$; 'na' indicates a lack of interaction term or alternate method for obtaining p-values. Soil type was not tested for the sown seeds. Distribution refers to which probability distribution was used for each model. For parameters (β and 95% CI) of each model see Appendix 4-15 to 4-18.

		Soil			Location			Soil: Location		
Measures of Success	Distribution	F-value	Stat	p-value	F-value	Stat	p-value	F-value	Stat	p-value
Adult Plants										
Survival (%)	Binomial	na	**0.11**	**0.744**	na	**3.50**	**0.321**	na	na	na
Proportion Bloomed	Binomial	na	**0.82**	**0.364**	na	**0.68**	**0.89**	na	na	na
Maximum Height (cm)	Gaussian	2.10	na	0.150	3.48	na	0.068	na	na	na
Number of Buds	Poisson	na	17.61	<0.001*	na	1.78	0.740	na	na	na
Total Seeds	Poisson	na	**5.24**	**0.800**	na	**0.80**	**0.370**	na	na	na
Proportion Full Seeds	Gaussian	1.49	na	0.229	1.16	na	0.372	3.7	na	0.018*
Seed Plot										
Seed Emergence (%)	Negative Binomial				45.97	25.98	<0.001*			
Seed Establishment (%)	Negative Binomial				51.21	26.69	<0.001*			
Seedling Height (cm)	Gaussian				1.08	na	0.410			

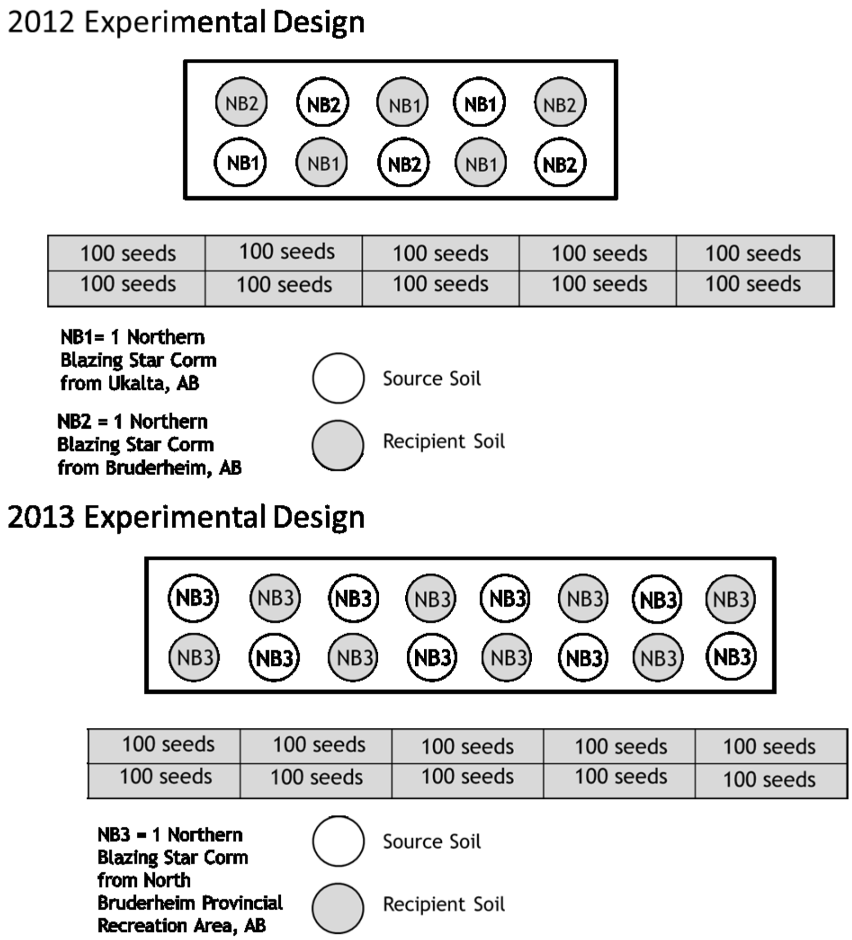
Table 4-3: Effect of *Liatris ligulistylis* 2012 corm size ($V=4/3\pi r^3$) on maximum height and number of buds for the 2013 and 2014 growing seasons. * indicates p-values <0.05

Variables	F-value	p-value
Maximum Height (2013)	0.69	0.413
Maximum Height (2014)	1.67	0.207
Number of Buds (2013)	0.57	0.458
Number of Buds (2014)	8.78	0.007*

Table 4-4: Mean annual temperatures (MAT), mean annual precipitation (MAP), and growing season (May-August) temperatures and precipitation in 2014 for the four translocation locations of *Liatris ligulistylis.* Percent normals refer to growing season temperatures and precipitation compared to 30 year normals data (1981-2010) for the month of May to August. Monthly temperatures were taken from weather stations in Fort McMurray, Lac La Biche, Woodbend and Brooks for the far north, north, central and south sites, respectively. For 30 year normals, weather stations data from Fort McMurray, Woodbend and Brooks (only precipitation) were used. Lac La Biche and Brooks lacked long-term temperature normals. Normals were used from nearby weather stations in Athabasca and Vulcan.

Location	MAT (°C)	MAP(mm)	Growing season temperature (°C) (May-August 2014)	Percent of Normal (1981-2010) for 2014 Growing Season Temperatures	Growing Season Precipitation (mm) (May-August 2014)	Percent of Normal (1981-2010) for 2014 Growing Season Precipitation
South	3.9	330	15.6	105.3	186.3	100.3
Central	2.7	384	14.4	102.7	218.5	71.3
North	1.9	416	14.8	104.6	234.1	78.5
Far North	0.0	384	14.8	103.9	244.6	98.8

4.7 Figures



100 seeds	100 seeds	100 seeds	100 seeds	100 seeds
100 seeds	100 seeds	100 seeds	100 seeds	100 seeds

100 seeds	100 seeds	100 seeds	100 seeds	100 seeds
100 seeds	100 seeds	100 seeds	100 seeds	100 seeds

Figure 4-1: Experimental design for 2012 and 2013 translocation sites. For the three translocation sites established in 2012, 10 *Liatris ligulistylis* adult plants were placed at each site, five from each of two source areas: Ukalta (NB1) and Bruderheim (NB2). For the nine translocation sites established in 2013, 16 *Liatris ligulistylis* adult plants were planted, all from the North Bruderheim Provincial Recreational Area (NB3). Plants were randomly allocated to one of two soil types: source soil from the collection site (white circle) or recipient soil from the translocation site (grey circle). Seed plots were established at both the 2012 and 2013 sites in 2013 with seed collected from the North Bruderheim Provincial Recreational Area. Seeds were sown onto raked recipient soil in 10 rectangular quadrats (30 cm by 30 cm). 100 seeds were placed in each quadrat at each site (i.e., 1000 seeds per site).

Figure 4-2: *Liatris ligulistylis* translocation site established in 2012 near Lac La Biche, Alberta.

Figure 4-3: *Liatris ligulistylis* translocation site established in 2013, ~110 km north of Fort McMurray near McClelland Lake, Alberta

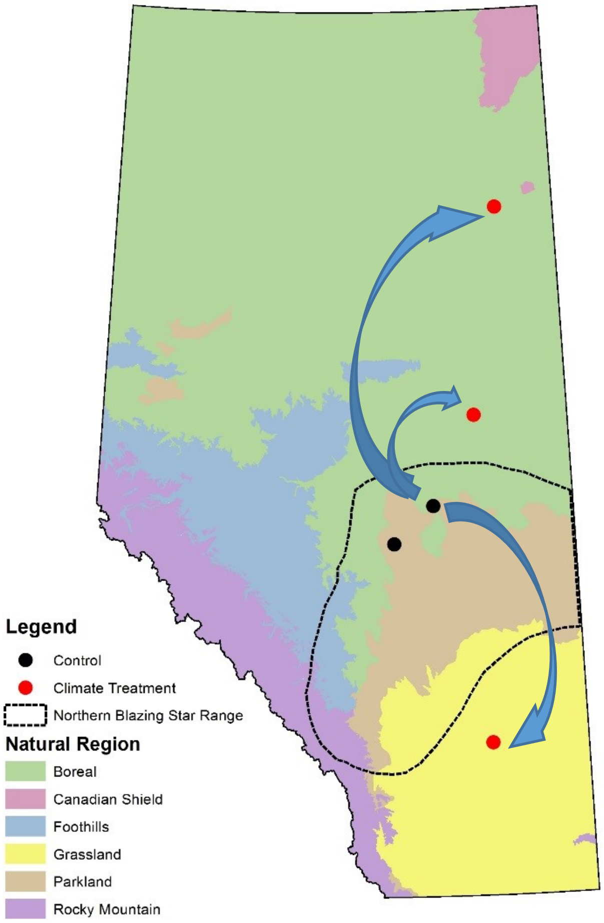


Figure 4-4: Map of Alberta indicating the geographical locations of *Liatris ligulistylis* translocation sites (established 2012-2013). Locations north of this species range were established to test the success of assisted migrations to higher latitudes. The south location was established to test this species' climate change vulnerabilities, as this area is predicted to be similar to the predicted future climate in this species' current range by the 2080s. All plots contained seed and adult *Liatris ligulistylis.* Base map from Natural Regions Committee (2006), with diagrams made with assistance from Amy Nixon (ABMI).

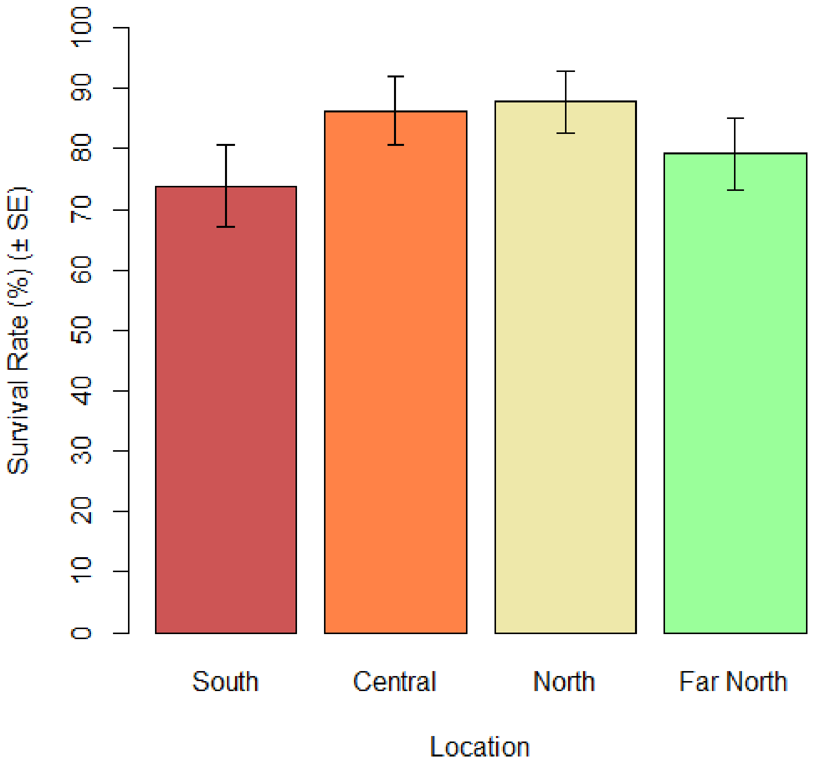


Figure 4-5: Survival rate (%) of adult *Liatris Ligulistylis* plants in 2014 at the four translocation locations. No significant difference in survival rates between locations.

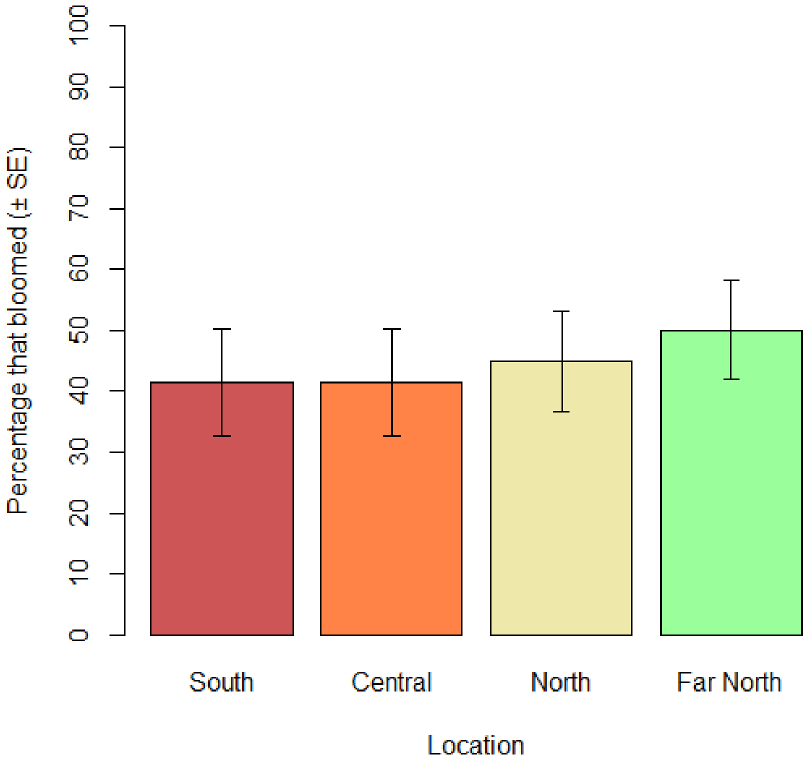


Figure 4-6: Percentage of adult *Liatris ligulistylis* plants which bloomed (i.e. produced flowering heads) in 2014 at the four translocation locations. No significant difference in the percentage of plants that bloomed between locations.

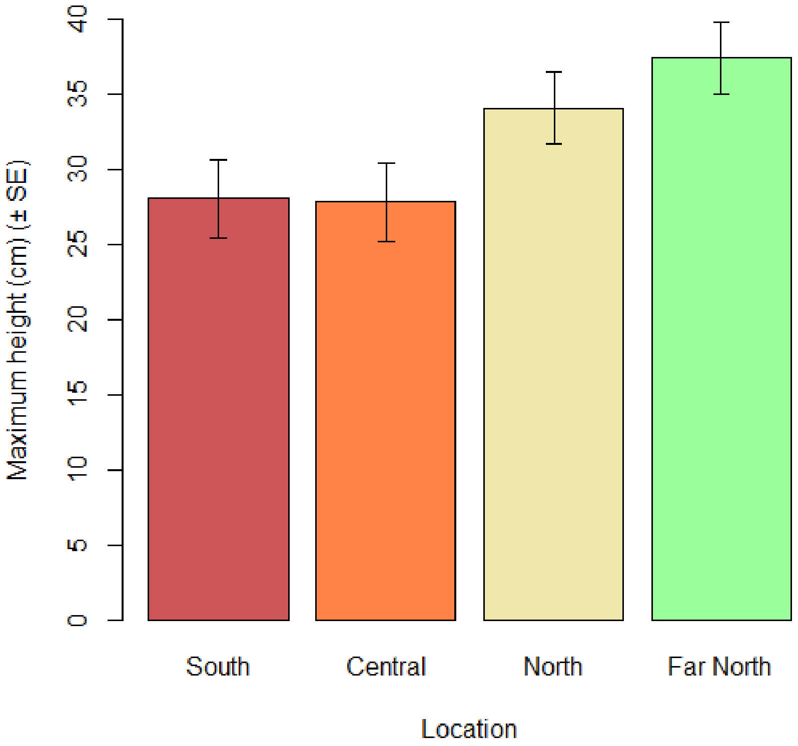


Figure 4-7: Maximum heights of *Liatris ligulistylis* adult plants in 2014 at the four translocation locations. No significant difference in maximum heights between locations.

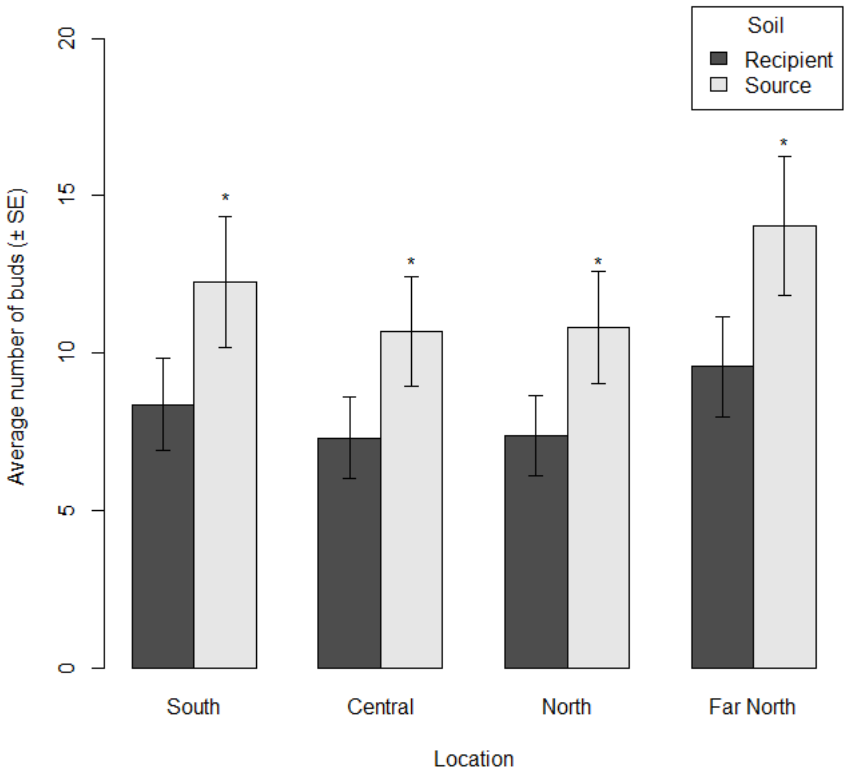


Figure 4-8: Average number of buds produced per *Liatris ligulistylis* plant in 2014 at each location by soil type (recipient or source). '*' indicates significant difference between soil types at a given location. There were no differences among locations and no location-by-soil interaction.

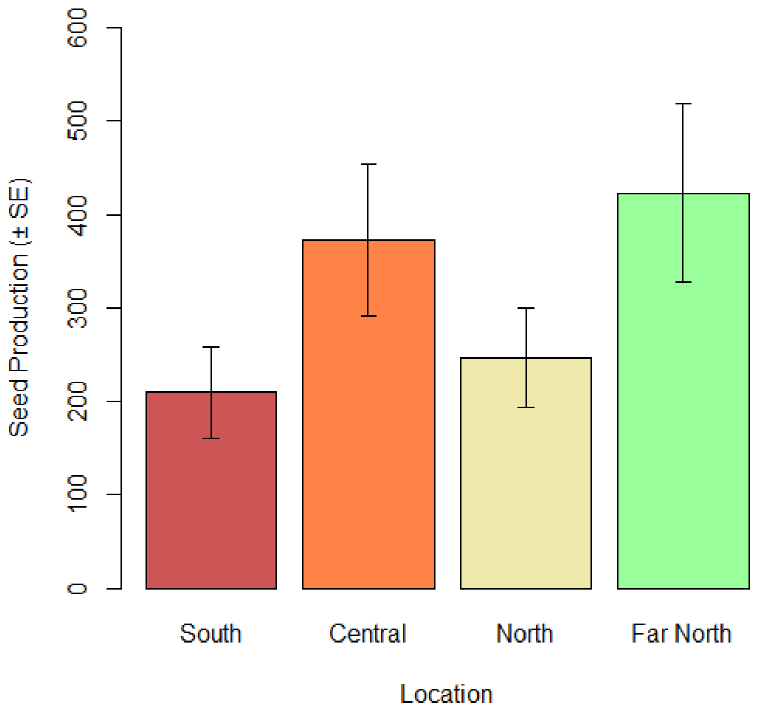


Figure 4-9: Seed production for adult *Liatris ligulistylis* plants in 2014 at the four translocation locations. No significant differences in seed production between locations.

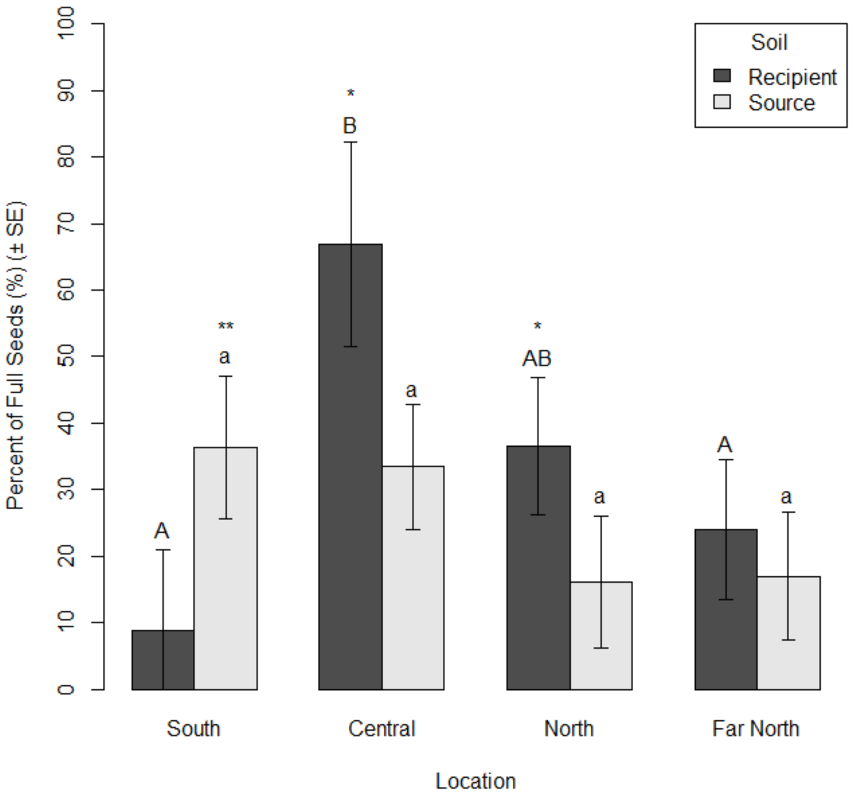


Figure 4-10: Percent of full seeds produced per *Liatris ligulistylis* plant in 2014 at each location by soil type (recipient or source). Different letters above bars indicate a significant difference between locations for plants in recipient soil (capital letters) or in source soil (small letters). "**" indicates significant difference ($p < 0.05$) between recipient and source soil at a given location. "*" indicates marginal significant difference ($0.05 > p < 0.1$) between recipient and source soil at a given location (see also Appendix 4-6 & 4-7).

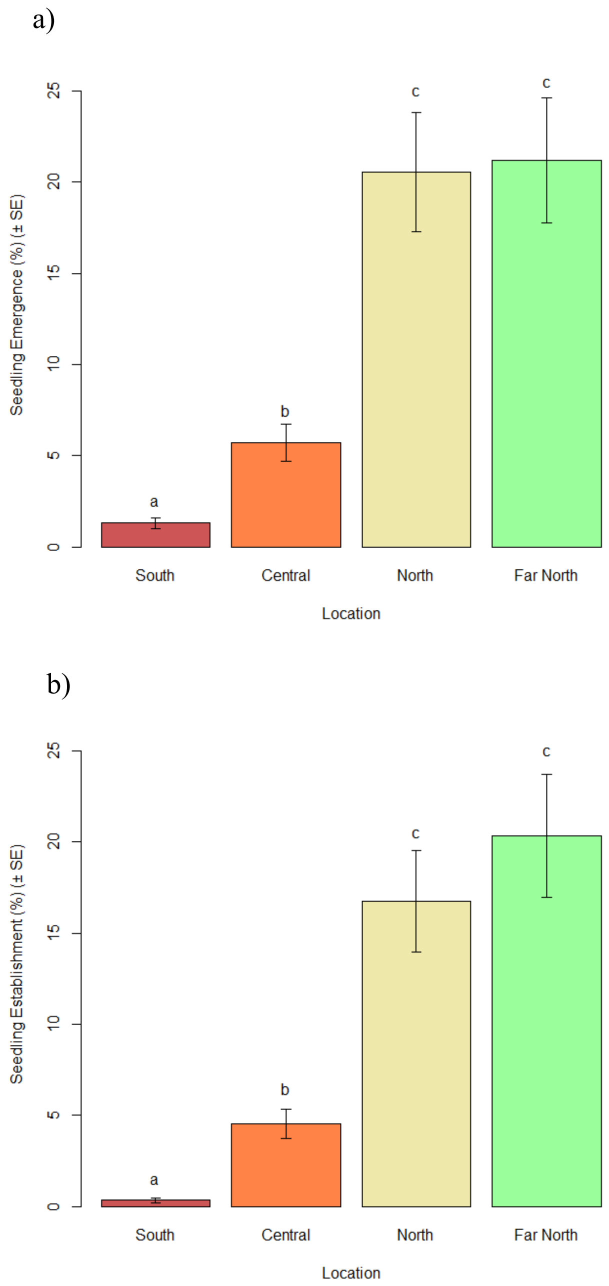


Figure 4-11: a) Seedling emergence for *Liatris ligulistylis* at the four translocation locations. Seedling emergence refers to the percent of seeds that germinated and emerged as seedlings between May – August 2014, after being sown in October 2013. b) Seedling establishment for *Liatris ligulistylis* at the four translocation locations. Percent establishment refers to the percent of seeds that germinated, emerged as seedlings between May-August 2014 and were still alive at the end of August 2014, after being planted in October 2013. Different letters indicate significant differences between locations based on post-hoc comparisons (see also Appendix 4-10, Appendix 4-11).

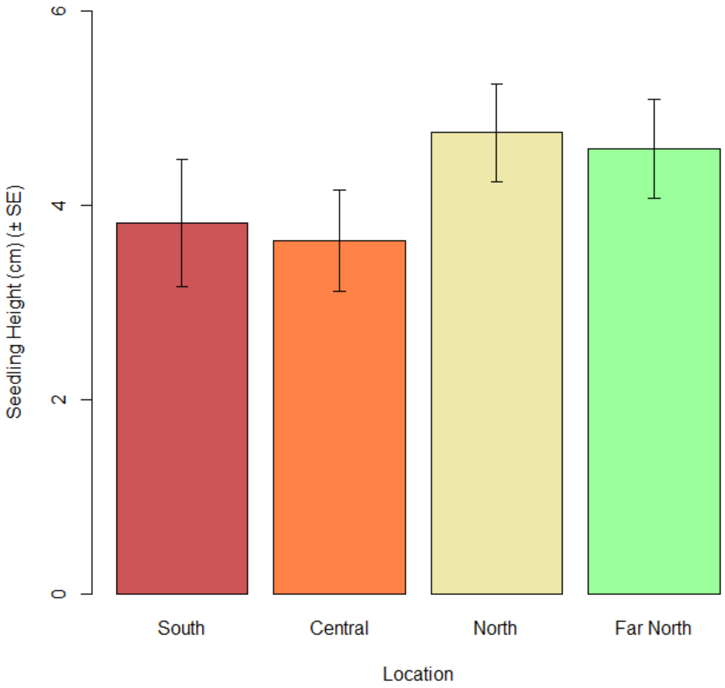


Figure 4-12: Seedling height for *Liatris ligulistylis* at the four translocation locations in 2014. No significant differences in seedling height between locations.

4.8 Appendices

Appendix 4-1: Summary of the 6 natural areas surveyed for *Liatris ligulistylis* including number of 500 m transects surveyed and whether the species was found.

Natural Areas	Latitude	Longitude	# Transects	*Liatris ligulistylis* present?
Northwest of Bruderheim Natural Area	53.852	-113.019	2	Y
North Bruderheim Provincial Recreational Area	52.869	-112.942	7	Y
Bellis Lake Natural Area	54.111	-112.170	4	Y
Halfmoon Lake Natural Area	54.052	-113.349	4	N
Redwater River Natural Area	54.076	-113.294	5	N
Opal Natural Area	53.987	-113.293	4	N

Appendix 4-2: Survey and sampling effort for *Liatris ligulistylis* in the North Bruderheim Provincial Recreational Area. Flowered refers to those plants which produced flowering stocks. Basal refers to those plants which did not produce a flowering stock. Browsed refers to those plants with evidence of browsing in the field.

Survey	
Total # of quadrats (1 m^2 each)	1215
Total # occupied quadrats	93
Total plants in 2013	273
Density of adult plants (# plants/m2)	0.22 (SE=0.05)
Sampling effort	
Date of Survey	August 9-23, 2013
Transect Length (m)	100
# Transects	61
Quadrat Interval (m)	5
Adult *Liatris ligulistylis* counts	
Flowered 2013	92
Basal 2013	174
Browsed 2013	7
Averages	
Number florets per plant	11.0 (SE=1.3)
Height of flowering plants (cm)	47.1 (SE=4.1)
Height of basal plants (cm)	17.2 (SE=1.7)
Height of browsed plants (cm)	22.3 (SE=2.8)

Appendix 4-3: Summary of canopy cover, bare ground and species composition from quadrats containing *Liatris ligulistylis* within the North Bruderheim Provincial Recreational Area. Survey was undertaken from August 9th-23rd 2013. Canopy cover refers to foliage above the individual plant.

Measurements	
Total occupied quadrats	93
Average Canopy cover (%)	0.7 (SE=0.26)
Average Bare ground (%)	16.7 (SE=1.50)
Species comp. (average % cover in quadrats)	
Trees (juvenile)	1.6 (SE=0.54)
Erect shrubs	13.1 (SE=1.29)
Forbs/Subshrubs	26.3 (SE=1.23)
Grasses/Sedges	23.8 (SE=1.48)
Bryophytes	15.3 (SE=1.77)
Lichens	1.6 (SE=0.71)
Dead wood	7.0 (SE=1.42)

Appendix 4-4: Summary of aspects and slope positions for occupied *Liatris ligulistylis* quadrats in North Bruderheim Provincial Recreational Area.

Aspect	Number of occupied quadrats
N=North	0
NE=Northeast	1
NW=Northwest	1
SE=Southeast	2
SW=Southwest	10
S=South	13
E=East	14
W=West	22
(-) = Flat	29
Slope	Number of occupied quadrats
C=Crest	5
Low=Lower Slope	8
Lev=Level	9
T=Toe	9
U=Upper slope	10
D=Depression	12
M=Middle Slope	39

Appendix 4-5: Post-hoc comparisons of maximum height (cm) of *Liatris ligulistylis* pairwise between the four translocation locations in 2014.

Contrast	Estimate	SE	DF	t-ratio	p
South - Central	0.23	3.67	8	0.064	0.999
South - North	-6.05	3.59	8	-1.683	0.391
South - Far North	-9.36	3.54	8	-2.645	0.110
Central - North	-6.28	3.61	8	-1.736	0.367
Central - Far North	-9.59	3.53	8	-2.717	0.099
North - Far North	-3.31	3.40	8	-0.975	0.767

Appendix 4-6: Post-hoc comparisons of the proportion of full seeds between soil types at each of the four translocation locations in 2014. '**' refers to p-value <0.05, '*'refers to p-value <0.1

Location	Contrast	Estimate	SE	DF	t-ratio	p
South	Recipient - Source	-0.28	0.13	45	-2.12	0.040**
Central	Recipient - Source	0.34	0.17	45	2.00	0.051*
North	Recipient - Source	0.20	0.11	45	1.89	0.065*
Far North	Recipient - Source	0.07	0.10	45	0.72	0.478

Appendix 4-7: Post-hoc comparisons of the proportion of full seeds pairwise between locations in 2014 with soil type kept constant as either recipient or source. '*' refers to p-value <0.05

Soil	Contrast	Estimate	SE	DF	t-ratio	p
Recipient	South - Central	-0.58	0.20	10	-2.97	0.014*
Recipient	South - North	-0.28	0.16	10	-1.74	0.113
Recipient	South – Far North	-0.15	0.16	10	-0.95	0.365
Recipient	Central - North	0.30	0.19	10	1.64	0.132
Recipient	Central – Far North	0.43	0.19	10	2.31	0.044*
Recipient	North – Far North	0.13	0.15	10	0.85	0.414
Source	South - Central	0.03	0.14	10	0.21	0.839
Source	South - North	0.20	0.15	10	1.39	0.195
Source	South – Far North	0.20	0.14	10	1.35	0.206
Source	Central - North	0.17	0.14	10	1.27	0.233
Source	Central – Far North	0.17	0.13	10	1.23	0.247
Source	North – Far North	-0.01	0.14	10	-0.06	0.956

Appendix 4-8: Summary of first date of for sprouting and bud burst for the 12 *Liatris ligulistylis* translocation sites at the four locations in Alberta (South, Central, North, and Far North). Information at Lac La Biche Site 1 could not be obtained as the camera was destroyed by vandalism.

Sites	Location	First sprout	First bud burst
Duchess Site 1	South	May 20, 2014	July 13, 2014
Duchess Site 2	South	May 15, 2014	July 20, 2014
Duchess 2012 Site	South	May 6, 2014	July 21, 2014
Bruderheim Site 1	Central	May 25, 2014	July 20, 2014
Bruderheim Site 2	Central	May 22, 2014	July 24, 2014
Woodbend 2012 Site	Central	May 11, 2014	July 22, 2014*
Lac La Biche 2012 Site	North	May 19, 2014	July 18, 2014
Lac La Biche Site 1	North	Destroyed	Destroyed
Lac La Biche Site 2	North	June 1, 2014	August 1, 2014
Fort Mac Site 1	Far North	May 31, 2014	August 3, 2014
Fort Mac Site 2	Far North	June 4, 2014	July 28, 2014
Fort Mac Site 3	Far North	June 1, 2014	after July 29, 2014**

*Camera tipped over so first photo of bud burst may have been earlier than July 22, 2014

**Camera malfunctioned no pictures after July 29, 2014

Appendix 4-9: Summary of data on soil properties for the 12 *Liatris ligulistylis* translocation sites at the four locations in Alberta (South, Central, North, and Far North). **Bolded** text indicates soil from the source location in Bruderheim Provincial Recreational Area. (TOC = total organic carbon, TN = total nitrogen, EC= electrical conductivity). ND = below detection limit.

Sites	Location	TOC	TN	pH	EC (µS/cm)	Sand%	Clay%	Silt%	PO_4-P (mg/L)	K (mg/L)
Duchess Site 2012	South	1.56	0.07	8.29	194	25.48	9.39	65.14	0.38	17.44
Duchess Site 1	South	1.45	0.12	6.57	73.1	32.08	16.04	51.88	0.49	20.40
Duchess Site 2	South	1.06	0.08	6.87	64.4	37.51	18.09	44.40	0.62	13.42
Bruderheim Site 1	**Central**	**0.87**	**0.02**	**5.69**	**41.9**	**14.64**	**10.65**	**74.71**	**2.42**	**5.13**
Bruderheim Site 2	**Central**	**1.01**	**0.03**	**5.69**	**34.8**	**14.66**	**11.99**	**73.35**	**1.50**	**3.26**
Woodbend/Devon Site	Central	0.98	0.06	8.11	94.8	20.00	15.33	64.67	0.41	7.93
Lac La Biche Site 2012	North	0.52	ND	6.08	23.5	12.82	11.39	75.79	5.61	1.95
Lac La Biche Site 1	North	1.29	0.03	5.61	40.2	15.73	11.44	72.82	5.87	3.82
Lac La Biche Site 2	North	0.47	ND	5.77	22.5	14.32	12.89	72.79	4.15	1.88
Fort Mac Site 1	Far North	0.45	ND	5.81	26.5	15.71	11.43	72.86	3.17	2.26
Fort Mac Site 2	Far North	0.62	ND	5.59	25.7	14.29	11.43	74.28	4.50	2.28
Fort Mac Site 3	Far North	0.51	ND	5.59	25.5	14.28	11.42	74.30	2.67	1.60

Appendix 4-10: Post-hoc comparisons of seed emergence between the four translocation locations in 2014. Seed emergence refers to those seed that germinated and emerged as seedlings between May-August 2014, after being planted in October 2013. '*' refers to p-values <0.05.

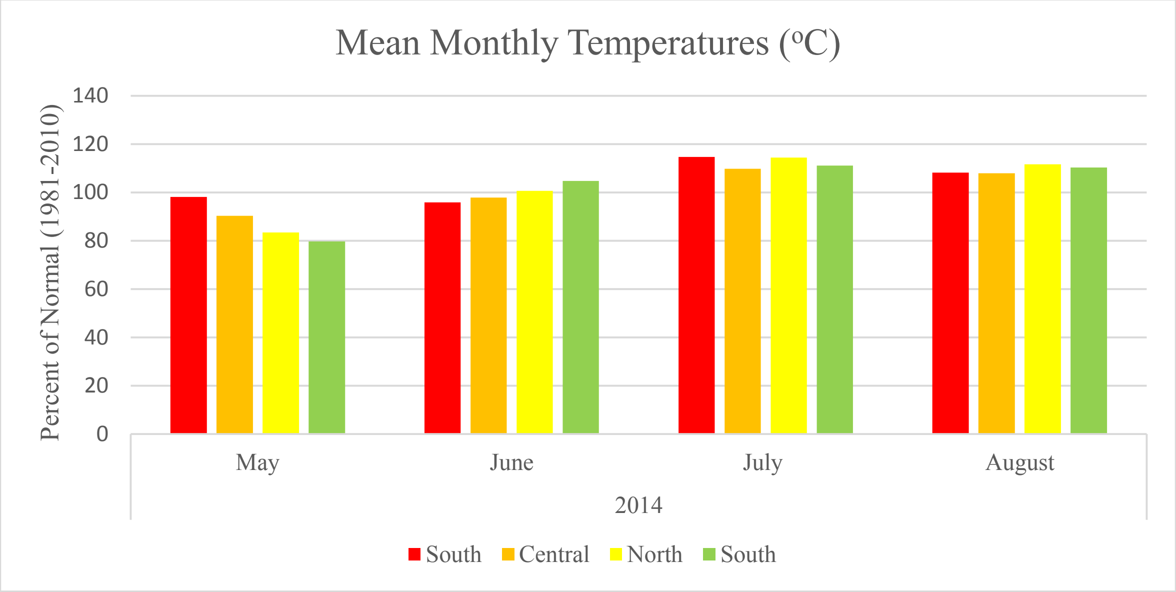
Contrast	Response ratio	SE	Z value	p
Central - South	0.23	0.06	-5.21	<0.001*
North - South	0.06	0.02	-10.11	<0.001*
Far North - South	0.06	0.02	-10.22	<0.001*
North - Central	0.28	0.07	-5.33	<0.001*
Far North - Central	0.27	0.06	-5.51	<0.001*
Far North - North	0.97	0.22	-0.14	0.999

Appendix 4-11: Post-hoc comparisons of seed establishment between the four translocation locations in 2014. Seed establishment refers to those seeds that germinated, emerged as seedlings between May-August 2014 and were still present at the end of August 2014, after being planted in October 2013. '*' refers to p-values <0.05.

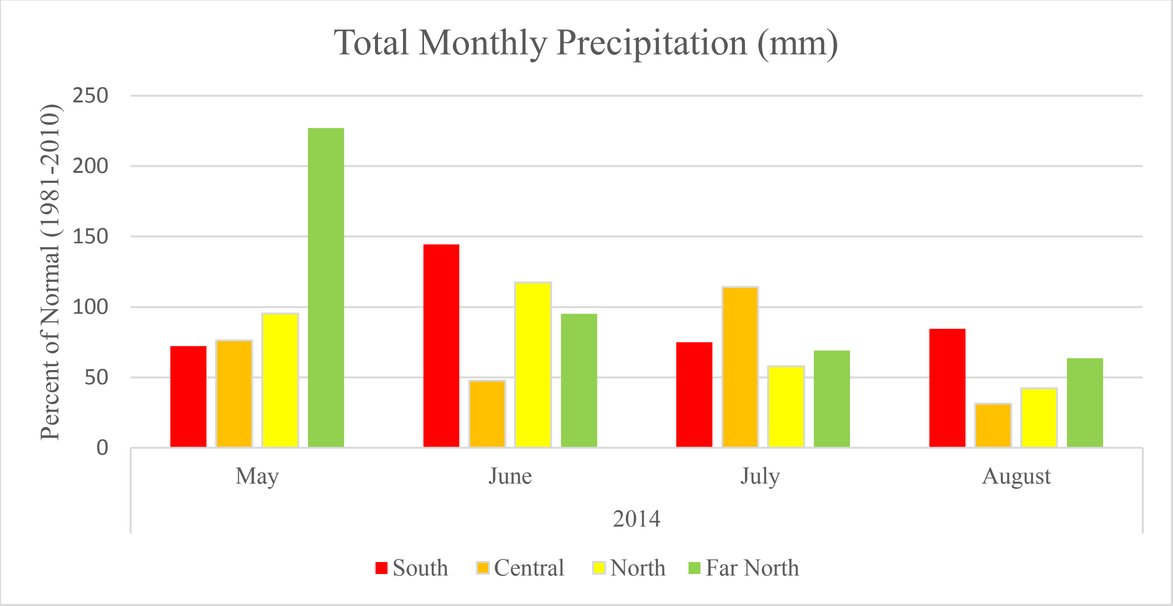
Contrast	Response ratio	SE	Z value	p
Central - South	0.07	0.03	-6.54	<0.001*
North - South	0.02	0.01	-9.99	<0.001*
Far North - South	0.02	0.01	-10.49	<0.001*
North - Central	0.27	0.07	-5.30	<0.001*
Far North - Central	0.22	0.06	-6.09	<0.001*
Far North - North	0.83	0.19	-0.82	0.846

Appendix 4-12: Re-count data for *Liatris ligulistylis* full seeds. Percent Error refers to the difference between the first (Count 1) and second (Count 2) counts.

Site	Plant ID	Count 1	Count 2	Percent Error (%)
Bruderheim Site 1	446	76	76	0.00
Bruderheim Site 2	547	123	122	0.82
Fort McMurray Site 2	460	209	209	0.00
Average				0.27 (SE=0.27)



Appendix 4-13: Average monthly temperatures during summer 2014 as a percent of normal (1981-2010) at the four translocation locations based on data from Environment Canada. Monthly temperature data for 2014 were taken from weather stations in Fort McMurray, Lac La Biche, Woodbend and Brooks for the far north, north, central and south sites, respectively. For the 30 year normals, the Fort McMurray and Woodbend weather stations were used, however due to a lack of normal data, weather stations in Athabasca and Vulcan had to be used for the north and south sites, respectively.



Appendix 4-14: Total monthly precipitation during summer 2014 as a percent of normal (1981-2010) at the four translocation locations based on data from Environment Canada. Monthly total precipitation data for 2014 were taken from weather stations in Fort McMurray, Lac La Biche, Woodbend and Brooks for the far north, north, central and south sites, respectively. For the 30 year normals, the Fort McMurray, Woodbend and Brooks weather stations were used, however due to a lack of normal data the Athabasca weather station had to be used for the north location.

Appendix 4-15: Summary of model parameters (β and 95% Confidence Interval) for survival, proportion bloomed, and maximum height. Intercept refers to the central location and source soil.

	Survival			Proportion Bloomed			Maximum Height		
Parameters	β	Lower CL	Upper CL	β	Lower CL	Upper CL	β	Lower CL	Upper CL
Intercept	1.78	0.92	2.64	-0.18	-0.85	0.49	30.60	25.78	35.42
Recipient Soil	0.14	-0.68	0.96	-0.33	-1.06	0.40	-5.56	-10.75	-0.37
South Location	-0.81	-1.97	0.35	0.00	-1.00	1.00	0.23	-6.98	7.44
North Location	0.13	-1.18	1.44	0.14	-0.84	1.12	6.28	-0.82	13.38
Far North Location	-0.51	-1.67	0.65	0.34	-0.62	1.30	9.59	2.67	16.51

Appendix 4-16: Summary of model parameters (β and 95% Confidence Interval) for number of buds and total seed production. Intercept refers to the central location and source soil.

	Number of Buds			Total Seeds		
Parameters	β	Lower CL	Upper CL	β	Lower CL	Upper CL
Intercept	2.37	2.06	2.68	5.99	5.56	6.42
Recipient Soil	-0.38	-0.56	-0.20	-0.58	-1.20	0.05
South Location	0.14	-0.31	0.59	-0.41	-1.01	0.19
North Location	0.01	-0.44	0.46	0.13	-0.49	0.74
Far North Location	0.27	-0.16	0.70	-0.12	-0.38	0.14

Appendix 4-17: Summary of model parameters (β and 95% Confidence Interval) for the proportion of full seeds. Intercept refers to the central location and source soil.

	Proportion of Full Seeds		
Parameters	β	Lower CL	Upper CL
Intercept	0.33	0.15	0.52
South Location	0.03	-0.25	0.31
North Location	-0.17	-0.44	0.09
Far North Location	-0.17	-0.43	0.10
Recipient Soil	0.34	0.01	0.66
South Location: Recipient Soil	-0.61	-1.03	-0.20
North Location: Recipient Soil	-0.13	-0.52	0.26
Far North Location: Recipient Soil	-0.26	-0.65	0.12

Appendix 4-18: Summary of model parameters (β and 95% Confidence Interval) for seedling emergence, seedling establishment and seedling height. Intercept refers to the central location and source soil.

	Seedling Emergence			Seedling Establishment			Seedling Height		
Parameters	β	Lower CL	Upper CL	β	Lower CL	Upper CL	β	Lower CL	Upper CL
Intercept	1.74	1.39	2.10	1.51	1.15	1.87	3.64	2.62	4.65
South Location	-1.49	-2.04	-0.93	-2.61	-3.39	-1.83	0.18	-1.45	1.82
North Location	1.28	0.81	1.75	1.31	0.82	1.79	1.11	-0.30	2.53
Far North Location	1.31	0.85	1.78	1.50	1.02	1.98	0.95	-0.47	2.37

Chapter 5: General Discussion

With increased levels of habitat fragmentation and the rate of climate change, new conservation strategies are needed to prevent the loss of rare and range-restricted species (Hoegh-Guldberg et al., 2008; Schwartz et al., 2012). One of these conservation strategies is assisted migration, which involves the movement of organisms to ranges that are predicted to be more suitable under future climate change scenarios (McLachlan et al., 2007; Loss et al., 2011). Although humans have been moving organisms for centuries (Vitt et al., 2010), experimental trials of assisted migrations that focus on mitigating the effects of climate change are still rare in scholarly literature (Hewitt et al, 2011). Future trials are therefore needed to gain a better understanding of this conservation tool. To improve the success of these assisted migrations a better understanding of the species' biology is needed before translocations occur (Gallagher et al., 2015). This may prevent issues of invasiveness (Ricciardi and Simberloff, 2009), but also dictate those methods that are likely to improve a species success at recipient sites (Chauvenet et al., 2012).

The main objectives of this thesis were to better understand the biology of two rare and range-restricted species in Alberta and to incorporate those understandings into the development of assisted migration conservation strategies. This thesis provided a greater understanding into the reproductive biology of *Houstonia longifolia*, and also preliminary results and methods from assisted migrations of *Houstonia longifolia* and *Liatris ligulistylis.*

5.1 Reproductive ecology of *Houstonia longifolia*

In order to formulate appropriate conservation strategies, the reproductive strategy of those species at risk needs to be understood. Therefore, I conducted an investigation into the reproductive ecology of *Houstonia longifolia*, including examination of morph biases and those factors affecting seed production. Morph ratios of *Houstonia longifolia* were hypothesized to be biased towards pins in small populations, given that pins usually have lower self-incompatibility than thrums (Ganders, 1979), and that these small populations were more prone to "demographic stochasticity and genetic drift" (Van Rossum et al., 2006). Results met these expectations with morph ratios in small populations being more pin biased, while larger populations tended to be more even to thrum biased. This suggests that that this species' pin floral morphs may be more self-compatible than thrum floral morphs.

From the univariate analysis of factors affecting seed production, no significant differences in between morph types was found, although seed from pin morphs had significantly higher germination rates than seed from thrums. Population size and local density were not related to seed production, while maximum height was positively related to seed production. Seed production varied by morph type and proportion pin plants with increases in the surrounding proportion of pin related to declines in seed production for pins, but increases in seed production for thrums. This provides evidence of a self-incompatibility system requiring the presence of opposite morph types for development of higher seed production.

Based on these above results, if assisted migrations are used for the conservation of *Houstonia longifolia* then equal proportion of pins and thrums should be planted at recipient sites in order to promote intermorph crosses. Although tall plants were positively related to seed production, stem height will vary between years; therefore targeting taller plants for translocation is unlikely to be useful in promoting future seed production at recipient sites.

5.1.1 Future research and limitations

One limitation of this study is that it occurred only over one growing season. Morph ratios may change over time and therefore continued monitoring should occur to determine the potential fluctuations in these ratios. Another limitation is the lack of understanding of potential niche differentiation between morph types (Kery et al., 2003). The morph types found within a site may be influenced by environmental factors such as temperature and soil. Therefore, future studies should investigate these and other environmental factors, which may be more favorable to one morph than the other, leading to changes in morph ratios over time through natural selection. In this study, pollinator intermorph visitation rates were not studied, but were believed to increase for large populations and dense patches, with intramorph visitation rates increasing in small populations (pin x pin) (Jacquemyn et al., 2002; Kery et al., 2003, Shibayama and Kadono, 2003). Also, it was believed that pollen in intermorph crosses would be more frequently moved from thrums to pins, instead of from pins to thrums (Van Rossum et al., 2006). Therefore, future pollination studies are needed to determine the rate of inter-versus intra-morph crosses; whether there is an asymmetric transfer of pollen between morph types (i.e. pins being better pollen recipients) (Van Rossum et al., 2006), and how pollination rates differ with populations of different sizes, local densities and proportions of pin plants. Future research should also investigate other factors affecting seed production in *Houstonia longifolia* in order to improve our understanding of limitations.

5.2 Assisted migration trials

Assisted migration trials of *Houstonia longifolia* involved equal proportion of pins and thrums being placed at each site. From these trials, only 8 of the 130 translocated plants survived to the end of June 2015, just one year after translocation. This most likely was a result of the dry weather conditions in the spring and early summer of 2015, with most translocation sites receiving less than half of their normal precipitation. Yearly weather fluctuations will always occur, and therefore continual assistance of plants and seed after their translocations may be necessary to ensure their survival (Early and Sax, 2011). This may include watering in drought years to increase the rates of this species establishment. No seedling emergence or establishment was observed for *Houstonia longifolia*. Earlier spring or prior fall sowing of seeds may have been more successful. Future translocations of seed should therefore consider early spring seed sowing where conditions are expected to be wetter; or if conditions are unfavourable then translocations may need to be done repeatedly to ensure more favourable years or when extensive droughts occur that they be assisted through continual watering and experimentation with shade tents.

The assisted migration of *Liatris ligulistylis* proved to be more successful than the assisted migration of *Houstonia longifolia*. Based on measurements collected in the summer 2014, translocated adult *Liatris ligulistylis* plants grew well hundreds of kilometers north of its current range; in these locations they also produced flowering stocks and seed in levels similar to those in the species' current range. The seed emergence and establishment rates were also significantly higher in the northern translocation sites than that south of its range or within its current range. This suggests that *Liatris ligulistylis* may have climate vulnerabilities and may be out of equilibrium with the climate in its current range. However, this study only occurred over one year. Weather conditions may have affected seedling establishment and survival. Nevertheless, this species may be an ideal candidate for future assisted migrations.

Based on experimental trials comparing source to recipient soils, future assisted migrations of *Liatris ligulistylis* should incorporate source soil, as those adult plants placed in their source soil produced a significantly higher number of buds and full seeds (south location) than those plants placed in recipient soil. This species is believed to have vesicular-arbuscular mycorrhizae associations, similar to those in *Liatris spicata*. By using source soil the appropriate mycorrhizae were most likely transplanted along with the plant (Medve, 1985; COSEWIC, 2012). Therefore, even if the recipient soil is judged to be fairly similar to the source soil, it may lack certain mutualistic organisms needed to promote a species' growth (McLachlan et al., 2007).

Differing root morphology may have led to fewer adult plants of *Houstonia longifolia* surviving than of *Liatris ligulistylis*. The fine fibrous roots of *H. longifolia* were more sensitive to transplanting than dormant corms of *L. ligulistylis*. Therefore, future trials should recognize the difficulty of translocating species with shallow fibrous root systems, and possibly use plugs grown in nurseries to overcome this weakness.

5.2.1 Future research and limitations

The main limitation of experimental assisted migration trials used here is that measurements were only recorded over one growing season (*Liatris ligulistylis* –May to August 2014, *Houstonia longifola* – May to July 2015). Weather conditions in each year likely affected the survival, growth, and reproduction of these species. Therefore, to determine the long-term success of assisted migrations, long-term monitoring of these sites will be necessary, along with translocations in different years. This study did not investigate mutualisms (i.e. mycorrhizal associations, pollinators), which may affect the success of these species. Although a mycorrhizal association is expected for *Liatris ligulistylis* (COSEWIC, 2012; Medve, 1985), further research is needed to determine if this relationship exists and the effect of this interaction on survival, growth and reproduction. Pollinator mutualisms should also be investigated to determine whether the pollinators at higher latitudes are sufficient and interested in *Houstonia longifolia* during the blooming of other species. The investigation of mutualisms should be conducted for any plants targeted for assisted migrations, as “paired” or “multispecies” translocations may increase the success of this conservation tool (McLachlan et al., 2007). Because bags were placed around *Liatris ligulistylis* to prevent its seed dispersal, the regeneration of this species from adult plants could not be investigated, although seed sowing from other plants proved successful. It is hoped that these assisted migration trials will lead to establishment of self-sufficient populations. Future research should therefore investigate the regeneration potential of these and other species after assisted migration. However, this regeneration research should only occur after a thorough investigation of the species’ invasiveness (Ricciardi and Simberloff, 2009).

Although large 2 gallon plastic pots were used in this study for translocation of plants, it is suggested that future assisted migration trials include biodegradable pots, in order to allow for a more natural integration of species into recipient sites. Also, the inclusion of enclosures should be used only for those species prone to browsing, as these enclosures were costly to establish, and may be impractical for large translocations containing numerous individuals. Finally, there is a general gap in knowledge of dispersal abilities for many plants (Royal Botanic Gardens KEW, 2015). This knowledge is necessary to provide guidelines on how quickly species could naturally move across the landscape, without multiple barriers from fragmentation and human development. By incorporating these dispersal capabilities into species distribution and habitat suitability modelling the selection of suitable recipient sites for assisted migrations will improve (McLachlan et al., 2006; Chauvenet et al., 2013). This investigation into dispersal capabilities could also help with prioritizing and selecting species for future assisted migrations (Barber et al., ‘in press’).

5.3 Prioritizing species and policy

Three criteria must be considered before assisted migration is used; this includes: 1) the extinction probability of the species, 2) the vagility of the species, and 3) the ecological role of the species (Hunter, 2007). Candidates for assisted migration should be at risk of extinction/extirpation due to climate change, have limited ability to colonize and disperse, and have minor ecological roles (Hunter, 2007; Early and Sax, 2011; Loss et al., 2011). Therefore, this tool is not appropriate for all species at risk of extinction and extirpation, with corridors and stepping stones still being the best option for long-dispersing species, with extensive climate ranges (Loss et al., 2011).

An investigation into the biology of species to be moved should be completed, not only to improve the success of this conservation tool, but also to prevent unwanted effects on recipient sites (Ricciardi and Simberloff, 2009; Chauvenet et al., 2012). However, this type of investigation is likely to be time-consuming and with the current pace of climate change, more trials are needed without a full knowledge of the species to be translocated (McLachlin et al., 2007). Therefore, the decision to use assisted migration may need to be determined by weighing the risks of this conservation tool (i.e. invasiveness) against the risks of extinctions (Chauvenet et al., 2013; Hoegh-Guldberg et al., 2008). However, Barber et al. (in press) has suggested a model to prioritize rare plant species in Alberta. This model provides a rapid assessment of species vulnerabilities by taking into consideration the velocity of climate change, habitat fragmentation and the dispersal abilities of rare plant species. Using this model the most vulnerable species could be prioritized for assisted migrations, even without a full understanding of these species' biology.

To improve the selection of species for assisted migration, multiple decision- making frameworks have been proposed, but a fully accepted one is still lacking (Hoegh-Guldberg et al., 2008; Richardson et al., 2009; Schwartz et al., 2012; McDonald-Madden et al., 2011). Therefore, policy makers, scientist and managers need to formulate a common framework which incorporates both social and scientific considerations (McLachlan et al., 2007, Chauvenet et al., 2013). The formulation of assisted migration policy is also necessary in order to promote the continuation of research trials and prevent "maverick" unsupervised translocations from local groups (McLachlan et al., 2007). As climate change progresses the need for this policy will increase in order to prevent numerous species from extinction (Thomas et al., 2004). With policy in place for translocations to higher latitudes, assisted migrations will move away from being considered a "last resort solution" and become accepted as a proactive conservation strategy (Chauvenet, et al., 2012).

5.4 Conclusion

This thesis provided a better understanding of the reproductive ecology of a locally imperiled distylous species *Houstonia longifolia*, and the potential use of assisted migrations as a proactive conservation tool. Although there is still much debate over the use of assisted migrations, these preliminary trials have given greater insight into the challenges associated with translocations of plants to higher latitudes, as well as the methods to be used in future trials. It is hoped that the results of this thesis will promote the formulation of appropriate conservation strategies for *Houstonia longifolia* and *Liatris ligulistylis*, as well as other rare and range-restricted species. In closing it is “[b]etter to maintain such species somewhere in the wild, rather than condemn them to ex-situ conservation or extinction” (Thomas, 2011).

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