

UNIVERSITY OF CALGARY

Development of Best Strategies for the Control of *Butomus umbellatus* L. (Flowering Rush) In  
Alberta

by

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A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES  
IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE  
DEGREE OF MASTER OF SCIENCE

GRADUATE PROGRAM IN BIOLOGICAL SCIENCES

CALGARY, ALBERTA

JANUARY, 2018

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## Abstract

The emergent aquatic perennial, *Butomus umbellatus* L. is a prohibited noxious weed in Alberta. In this thesis I provide (1) the first comprehensive review on its phenology and cytotype in Alberta; (2) an examination of the effect of cytotype on propagation means and (3) an experimental test of different control methods in two infested Alberta lakes. One of the findings of this study is that a full understanding of the propagation of this species is complicated because there are two cytotypes: fertile diploids and sterile triploids. I describe in this thesis how most populations of *B. umbellatus* in Alberta appear to be the diploid cytotype, except for a triploid population in Innisfail.

My studies found that in diploid plants, sexual reproduction is not the primary means of spread. My results also indicate that all control methods currently in use are equally unsuccessful, largely because *B. umbellatus* invests so heavily in vegetative reproduction that removing the entire rhizome is difficult. Because of this life history feature, *B. umbellatus* will likely be most effectively controlled by quickly recognizing new populations and removing all plant material. While revegetation of a reclaimed area with indigenous plants could prove beneficial, my results indicated that *B. umbellatus* quickly reclaims sites that have been replanted with native species.

## **Acknowledgements**

The author would like to thank her supervisor, Dr. Jana Vamosi for tirelessly reviewing the many versions of this thesis, committee members Drs. Leland Jackson and Steven Vamosi for their guidance, and the Western Irrigation District and Mitacs for their funding support.

Thank you as well to Rachel Redick for all her hard work in the field and in the lab, Bonita Smith in the U of C Herbarium for her plant identification know how, Diane White, U of C greenhouse manager and Mathew Quinn for his knowledge and enthusiasm for the project. Thank you to the people of Camp Koinonia at Lake Isle for their help and hospitality, especially Dave and Bunny Eidick, Kristine Buchholtz and Doug Buchholtz.

Work at Lake Isle would not have been possible without the help of Don Hare, Tanya Rushcall, Kate Wilson and Janelle. Thank you all for braving the Flowering Rush waters!

*For getting me to open my mind... Thanks G.B.*

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## Chapter One:

### **Biological invasions of aquatic communities: *Butomus umbellatus* L. in Alberta**

The International Union for Conservation of Nature (IUCN) considers invasive nonindigenous species to be, after habitat loss, the second most significant threat to biodiversity (IUCN, 2016). Newly introduced plant species are often free of predation and disease, as they are not recognized by local animals or pathogens and can proliferate in different environments via rapid evolution and superior competitive ability (Kohli et al. 2006, Whitney and Gabler, 2008). Upon their arrival to introduced communities, nonindigenous plants often exhibit elevated population growth compared to their indigenous neighbors (Da Silva and Sargent, 2011). When nonindigenous species reproduce more quickly than indigenous species to the extent that they cause displacement, these nonindigenous plants are categorized as invasive (White et al. 1993, Marsden and Hauser, 2009). It is important to note that the terms nonindigenous or non-native have societal implications. Garcia-Llorente et al. (2008) found the more recent a species' introduction was, or if an education campaign was in place, the more recognizable the species was as nonindigenous by the general populace. The longer a nonindigenous species had been present, the more likely it was accepted as part of the local ecosystem. Fossil records indicate that aquatic plant invasions occurred with regular frequency for thousands of years (Ashton and Mitchell, 1989). These natural invasions are usually sporadic, and their success is dependent on the plant species' ability to overcome many biotic and abiotic factors (Ashton and Mitchell, 1989). In 50 years, *B. umbellatus* has spread from the Great Lakes to most of the northern United States and southern Canada (CFIA, 2008) after deliberate transport. Like many other invasive species, *B. umbellatus* was introduced to Alberta about 30 years ago, as a result of horticultural escape. It is

observed to aggressively colonize a variety of habitats, including rivers, lakes, ponds, irrigation canals and culverts, thus earning its designation of nonindigenous invasive prohibited noxious weed (Kliber and Eckert, 2005).

This thesis is the result of a collaboration between industry (Mitacs Industry Partner, Western Irrigation District), academia and government (Alberta Invasive Species Council, Environment and Parks), and therefore reflects the aims of scientific inquiry as well as science communication to stakeholders and the public. Initial consultations with dozens of lakeside land owners at information symposiums in Alberta revealed how little was known about Flowering Rush, including how to identify it or how to control it. To attempt to provide basic information on *B. umbellatus* for all involved partners, this chapter continues with a literature review of its first introductions to Canada as well as its phenology and ecology. This literature review revealed that (1) *B. umbellatus* is a variable species in its phenology and (2) many different control methods are being used in Alberta with minimum coordination or quantitative assessment of their effectiveness.

*B. umbellatus* exhibits variation in ploidy (i.e., the number of sets of chromosomes), having diploid ( $2n=26$ ) and triploid ( $3n=39$ ) cytotypes, which could affect its seed production and rate of spread in Alberta (an examination of whether this ploidy variation is present in invaded lakes within Alberta forms the topic of Chapter Two). A controlled quantitative assessment of the common control methods was deemed necessary to determine the most effective way to control this invasive species (Chapter Three). The goal was to develop a richer understanding of the problem in the hopes of informing stakeholders and decision makers, as they form an effective management program (Garcia-Llorente et al. 2008).

## **1.1 *Butomus umbellatus* in Canada**

The first recorded presence of *B. umbellatus* in North America was along the shores of the St. Lawrence river near Montreal in 1897. It quickly spread into eastern Lake Ontario (Kliber and Echert, 2005) and today, *B. umbellatus* is found in most of the northern United States and all provinces in Canada, principally along the Canada/US border.

Observations during the initial phases of establishment reveal that *B. umbellatus* first appears in an area in scattered populations where there is then a local expansion of colonies with downstream spread (Staniforth and Frego, 1980). Muskrats and waterfowl appear to be a primary mode of dispersal once a plant arrives in an area (Staniforth and Frego, 1980). Their activities fragment rhizomes, dislodge bulbils and thereby disperse the species. It is currently unknown whether seeds (sexual reproduction), bulbils or rhizomes (clonal/vegetative reproduction) are the primary dispersal method when new colonies appear in isolated locales in Alberta.

In Canada there are two forms or cytotypes (karyotypes) of *B. umbellatus*: fertile diploids and sterile triploids. While much of *B. umbellatus* in Europe has been classified as the triploid cytotype, North American populations are more often diploid (Kliber and Eckert, 2005). The differences between allocation to seeds and vegetative dispersal structures between diploids and triploids can influence the mode of dispersal and appropriate control measures of this species (See Chapter Two).

## **1.2 Morphology and ecology of *Butomus umbellatus***

*Butomus umbellatus* is a monotypic member of the family Butomaceae. *B. umbellatus* is a perennial species indigenous to Eurasia and resembles a large sedge with vertical foliage that can reach 2m in height. Leaves are triangular in cross section, spongy and compressible and narrow

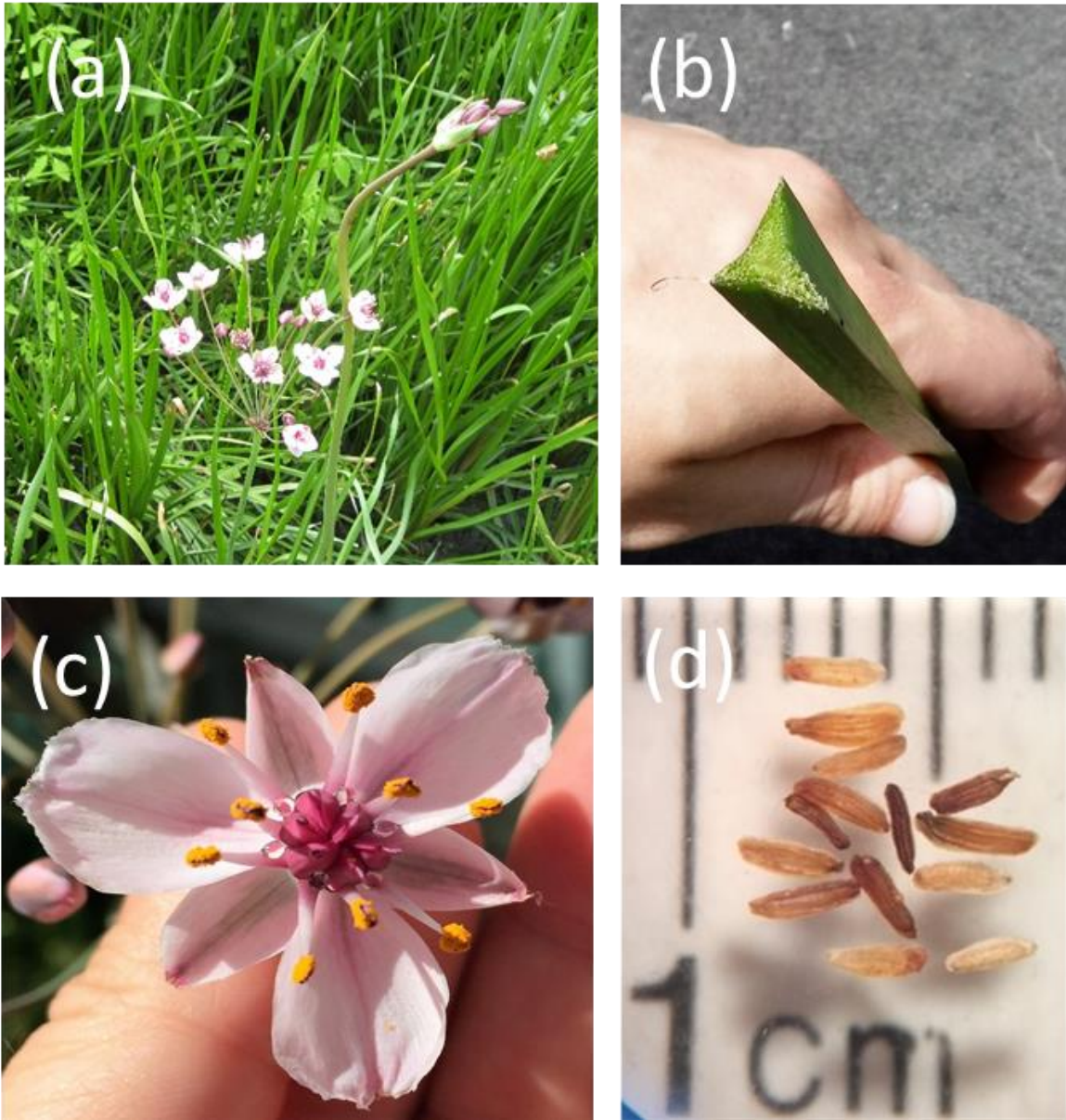
from base to tip. Leaves begin to spiral near the tips (Fig. 1.1a, b). When first emerging in the spring, the bases of leaves are usually dark red. Easiest to identify when flowering; *B. umbellatus* has 20-50 flowers growing in an umbel. Individual flowers have three petals and three sepals which are light pink to rose-coloured. Sepals are smaller and may be slightly greenish. Flowers are 2-2.5cm wide and have nine stamens arranged in an outer whorl of six and an inner whorl of three. There are six carpels, each of which can produce about 200 seeds (Fig. 1.1 d) (Parkinson, 2010). At the base of the carpels drops of nectar are produced (Fig. 1.1c), which is observed to attract bees, wasps, flies and members of Lepidoptera (e.g. butterflies) (Lui et al. 2005).

*B. umbellatus* can reproduce sexually and vegetatively, yet the relative contribution of each mode of reproduction varies between the two cytotypes (Table 1). Diploids are fully fertile and self compatible. Diploids generally have a lower overall production of biomass and produce fewer lateral rhizome buds (Hroudova et al. 1996). This fertile type of Flowering Rush has four different methods for reproduction. It can reproduce by vegetative bulbils on the rhizomes (Fig. 1.2a), fragmentation of the rhizomes (Fig 1.2b-c), seed production and inflorescent bulbils. Inflorescent bulbils have not been observed in Alberta. Rhizome bulbils are buoyant and represent a complete clone of the parent plant. Triploids are self incompatible and usually sterile, do not flower as often as diploids and typically reproduce via rhizome fragmentation only (Hroudova et al. 1996). Triploids also have a higher production of overall biomass, tend to be hardier and spread easily via vegetative propagation (Hroudova et al. 1996). Individual populations of *B. umbellatus* are usually represented by a single cytotype (Krahulcova and Jarolimov, 1993). Aneuploidy is also quite common in this species with recorded chromosome counts of 24, 26, 27, 28, 29, 30 and 40 (IPCN, 2017). The cytotype frequency in Alberta is currently unknown and its determination may

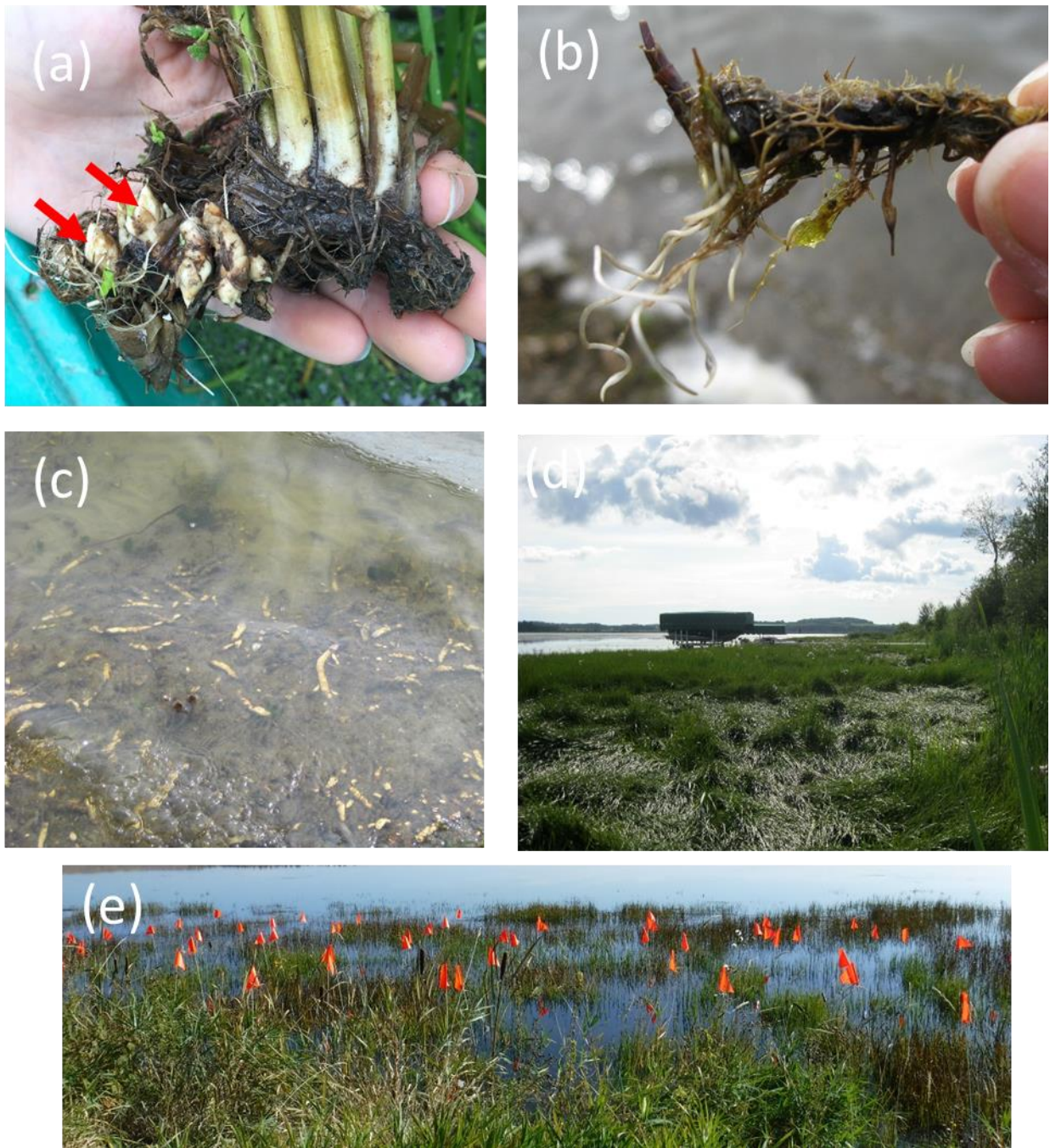
be critical to control this invasive species (Table 1) and therefore represents a main objective of this thesis (See Chapter Two).

**Table 1:** A summary of the growth habits of diploid and triploid *B. umbellatus* and the considerations for possible control methods.

<b>Diploids (2n=26)</b>	<b>Triploids (3n=39)</b>	
Fully fertile	Infertile	If seed producing, control methods must address both the flowers and the seed bank.
Less numerous rhizome buds, produces bulbils	High production of rhizome buds, little bulbil production	
Lower biomass produced	Higher biomass produced	Most of Alberta's lakes are eutrophic. Diploids produce massive amounts of biomass despite high nutrient inputs.
Habitats with more acidic and base-poor soils; less tolerant of high nutrient inputs	Habitats with less acidic and more base-rich soils; more tolerant of high nutrient inputs	



**Figure 1.1:** (a) *Butomus umbellatus* floral umbel, (b) cross section of the triangular stem, (c) single flower with 6 drops of nectar at the base of the six carpels, (d) seeds of *B. umbellatus*.



**Figure 1.2:** (a) Rhizome bulbils (arrows), (b) rhizome fragment with preformed roots, (c) bed of rhizomes on a lake bottom uncovered by winter ice, (d) collapse of mature plants, (e) new growth in October.

The rhizomes are fleshy and contain large carbohydrate reserves and preformed roots (Fig. 1.2b). *B. umbellatus* produces more underground biomass than above ground shoots and most of the rhizome biomass is produced late in the growing season (Hroudova et al. 1996). Individual plants have a monopodial rhizome that grows from the apical tip. The trailing portion of the rhizome dies off at the end of each growing season (Lui et al. 2004). Rhizomes can easily separate from the parent plant, a process facilitated by a constriction that develops between a bud and the main rhizome. This allows sections to break off easily with minor disturbances such as moving water, passing boats or waterfowl (Parkinson, 2010). The buoyancy of these fragments allows them to travel some distance before establishing a new plant population. Rhizomes are extremely hardy and can survive in a desiccated state for several weeks, sprouting within days of being placed in water. This hardiness is of concern because it increases the likelihood of unintentional transport of rhizomes between water bodies by boats.

The most appropriate methods of control may also depend on the conditions of the water body being invaded. *B. umbellatus* can survive at a variety of water depths; growing emergent from the shoreline up to fully submerged in waters as deep as 4.5m (Madsen et al. 2016). Water fluctuations promote the establishment and expansion of *B. umbellatus*, stimulating rhizome growth (Hroudova et al. 1996). A decrease in water levels exposes unvegetated or sparsely vegetated substrate that warms quickly when exposed to sunlight. These two conditions promote sprouting and accelerate the growth of rhizome budding or seed germination that leads to shoot multiplication and establishment of new plants (Hroudova et al. 1996). If water levels remain low the following growing season, the population of *B. umbellatus* can expand. Repeated exposure of the substrate makes the proliferation of *B. umbellatus* by vegetative propagation possible

(Hroudova et al. 1996). These features make this species particularly prone to invading irrigation canals and reservoirs that experience regular seasonal raising and lowering of waters.

In North America, rhizomes begin to sprout before indigenous vegetation (Parkinson, 2010). Flowering occurs from early summer to mid-fall. In contrast to the growth form of other emergent aquatic plant species like cattails, the leaves of *B. umbellatus* collapse (Fig 1.2d) and decompose. This process results in decreases in oxygen and increased total nitrogen and phosphorus levels (Li et al. 2014). The increased nutrient levels can lead to enhanced algal growth and increased water turbidity resulting in shading of other aquatic plant species (Dorgham, 2014). In the fall when the current season's growth is decomposing, a new generation of growth takes place until the first frosts when growth stops (Fig. 1.2e). Despite the halt in growth at this point of the season, seeds of indigenous species are prevented from reaching potential germination sites due to the thick stands of *B. umbellatus*, which likely accelerates the establishment and spread of *B. umbellatus*.

### **1.3 The impact of *Butomus umbellatus***

Invasive aquatic plant species like *B. umbellatus* adversely impact irrigation. In Alberta, 625,000 hectares of land in the province receive water via irrigation to support the agriculture, livestock and food processing industries (Alberta Water Portal, 2017). Water flow in irrigation canals can be slowed by dense stands of aquatic weeds (Bentivegna and Fernandez, 2005), while dislodged vegetation can clog trash racks and intake pumps (Alberta Agriculture and Forestry, 2016).

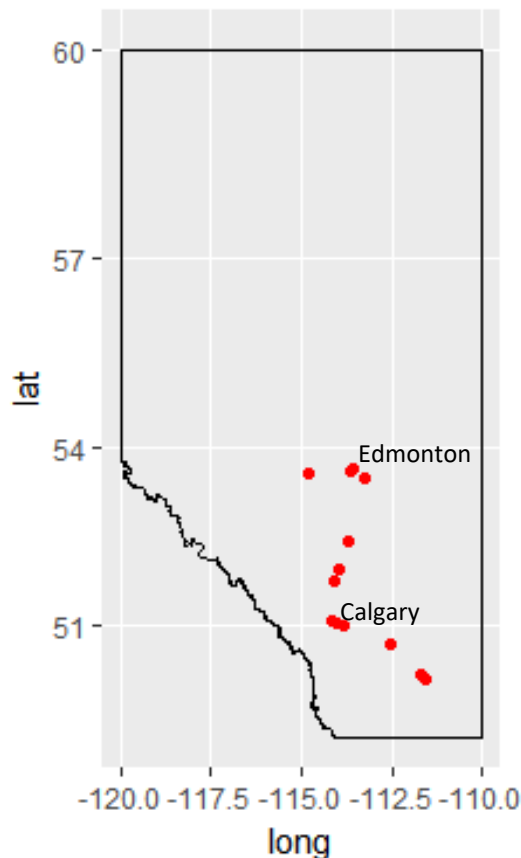
The societal impacts of *B. umbellatus* are perhaps more pronounced. It has become a significant problem for residents along shorelines and to recreational users of water bodies (Madsen et al. 2016). Dense and wide spread stands of Flowering Rush, has impeded open water

recreation in areas of Flat Head Lake, Montana (Rice and Dupuis, 2009) and threatens to do the same in Alberta lakes.

To date, various control methods for *B. umbellatus* that include: (1) cutting flower heads, (2) steaming the plants and (3) cutting plants back below the water line, have appeared to be ineffective at control. There is an ongoing study of the effectiveness of using an herbicide on plants in Innisfail but, thus far, results appear to be inconclusive (AFW, 2016). In a similar system in Montana (Flat Head Lake), herbicide trials have also failed to demonstrate this method of control is effective (Rice, et al. 2009). Previous studies have not measured how individuals or populations of *B. umbellatus* are affected by these control measures in terms of seed production, rhizome size, or density. This thesis will provide a quantitative comparison of control methods including introducing indigenous plants as a form of competitive control, benthic barriers and complete manual removal of plant material (See Chapter Three), as well as some observational studies on cutbacks, suction harvesting and further herbicide use (See Appendix D).

#### **1.4 *Butomus umbellatus* in Alberta with a focus on two aquatic systems**

Since its first recorded appearance in 1990 along the Sturgeon River, *B. umbellatus* can now be found in a variety of locations in Alberta (Fig. 1.3). There are approximately three hypothesised points of introduction throughout Alberta: The Sturgeon river; Buffalo Creek in Innisfail; and the Bow River region (Alberta Government, 2016). Within Alberta, two lakes are of interest due to their heavy recreational use: Isle Lake and Chestermere Lake. These two lakes comprise the study sites of this thesis and are detailed in the next section.



**Figure 1.3:** Locations of *B. umbellatus* in Alberta as of 2016 recorded by the Government of Alberta. *B. umbellatus* has been found in Isle Lake, the Sturgeon River in St. Albert, ponds in Sherwood Park and Lacombe, Buffalo Creek in Innisfail, ponds in Olds, and Calgary, the Bow River as far as Cypress County and in the headwaters canal from the Bow River in Calgary to Chestermere Lake.

#### 1.4.1 Isle Lake

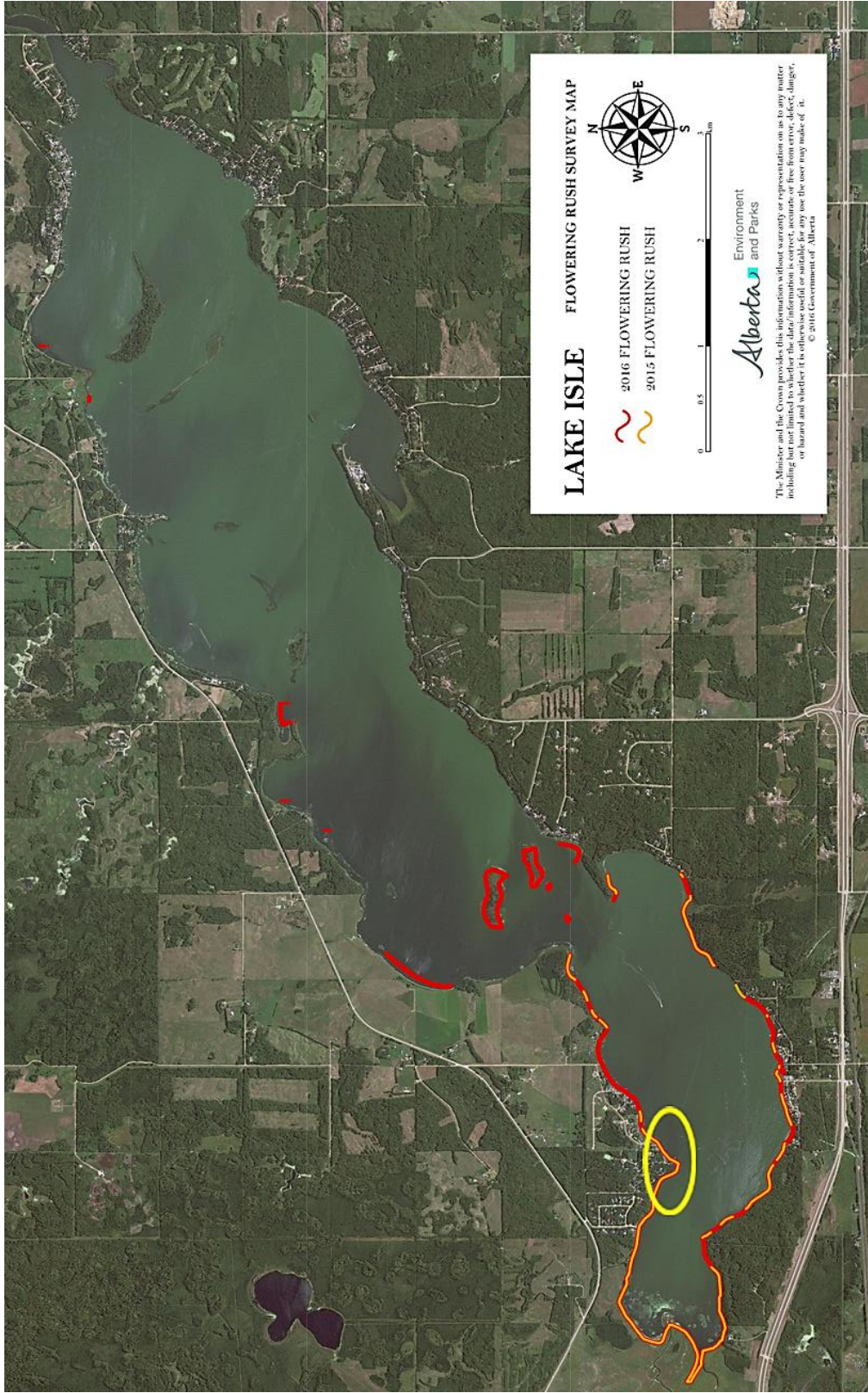
Isle Lake (or Lake Isle, locally) is a medium sized lake (total area of 23km<sup>2</sup>) 80km west of Edmonton. It is shallow with a maximum depth of 7.5m and an average depth of 4.1m. The lake is prone to fluctuations in water level which has varied as much as 1.5m between 1960 and 1987 (Mitchell and Prepas, 1990). The littoral zone comprises 40% of the lake's total surface area (Mitchell and Prepas, 1990).

The lake is thought to have been eutrophic for the last 4000 years, as determined through sediment core samples taken in 1977 (Mitchell and Prepas, 1990). Thus, algae growth is high and blue green algae blooms are common in the summer months (Mitchell and Prepas, 1990). Historically a popular fishing site, fish numbers and diversity have declined recently through a

combination of commercial fishing, which ended in 1972, winter kills and a changing lake ecology (Mitchell and Prepas, 1990).

Since its arrival to Lake Isle, *B. umbellatus* populations have spread rapidly from the initial introduction to the western basin. Between 2015 and 2016, the coverage of the lake by *B. umbellatus* increased from approximately 8km of shoreline to 15km of shoreline and is advancing eastward towards the lake's outflow (Fig. 1.4).

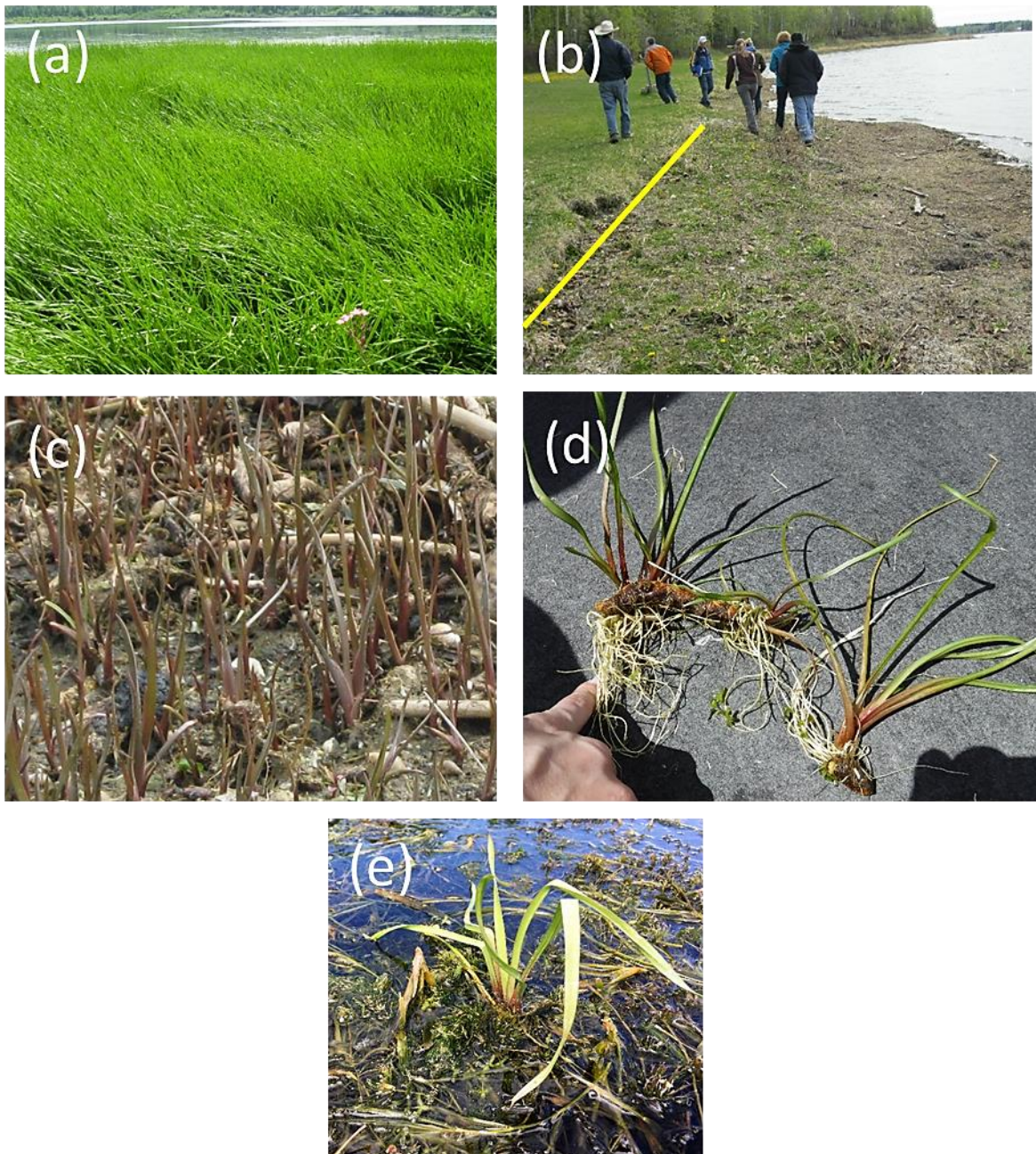
As *B. umbellatus* has colonized the lake, indigenous plant species have diminished, leaving monotypic stands of Flowering Rush along the shores, especially in the western basin (Fig. 1.5a). An inventory of the species of terrestrial and aquatic plants other than *B. umbellatus* that can be found at Lake Isle within the experimental site was conducted by myself (see Appendix A). Voucher specimens were pressed, dried and submitted to the University of Calgary Herbarium for identification by herbarium technician Bonnie Smith.



**Figure 1.4:** (Previous page) Surveys conducted in 2015 and 2016 by Alberta Environment and Parks show the spread of *B. umbellatus* across Lake Isle. Site of experimental trials is circled (Reprinted with permission from Alberta Environment and Parks, 2017).

Mats of decayed vegetation trap sediment and other debris, creating a new shoreline which has slowly been advancing out into the lake (Fig. 1.5b). Rhizomes can grow out of last year's decomposing plants without attaching to the underlying substrate (Fig. 1.5c) and can grow out of floating mats of aquatic plants (Fig. 1.5e).

Some lake residents have removed Flowering Rush stands near their property, which contributed to the rapid spread of Flowering Rush through the release of bulbils or rhizome fragments (Fig. 1.5d). In an effort to communicate the biology of this plant, I produced a summary brochure to encourage dialogue and disseminate information (See Appendix E).



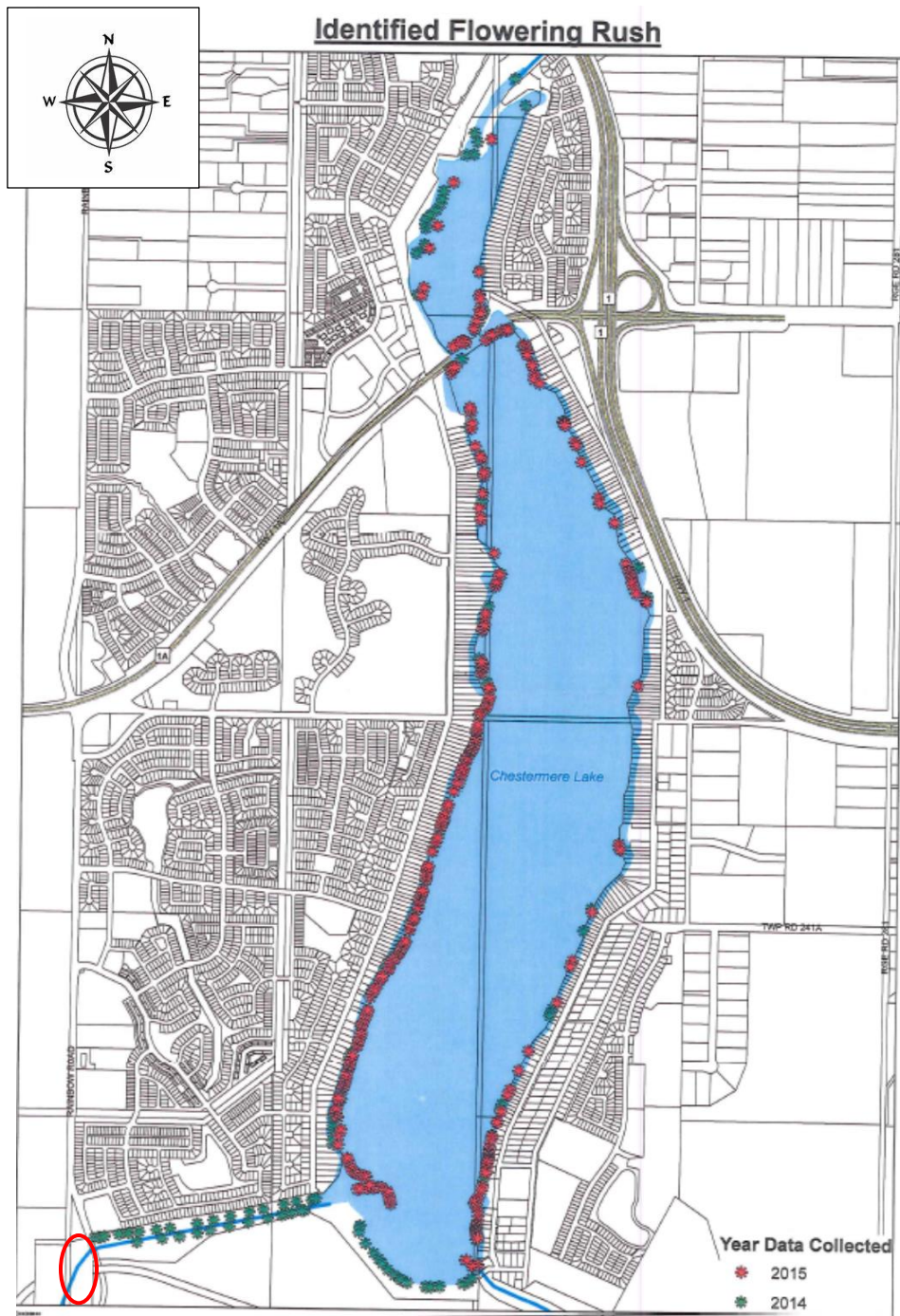
**Figure 1.5:** (a) Monotypic dominant stand of *B.umbellatus*, (b) original shore line (line) expanding from mats of decayed *B. umbellatus*, (c) *B. umbellatus* growing out of the previous year's decomposing material (d) rhizome fragments which have sprouted into new, complete plants (e) *B. umbellatus* growing out of a floating mat of other aquatic plants.

### 1.4.2 Chestermere Lake

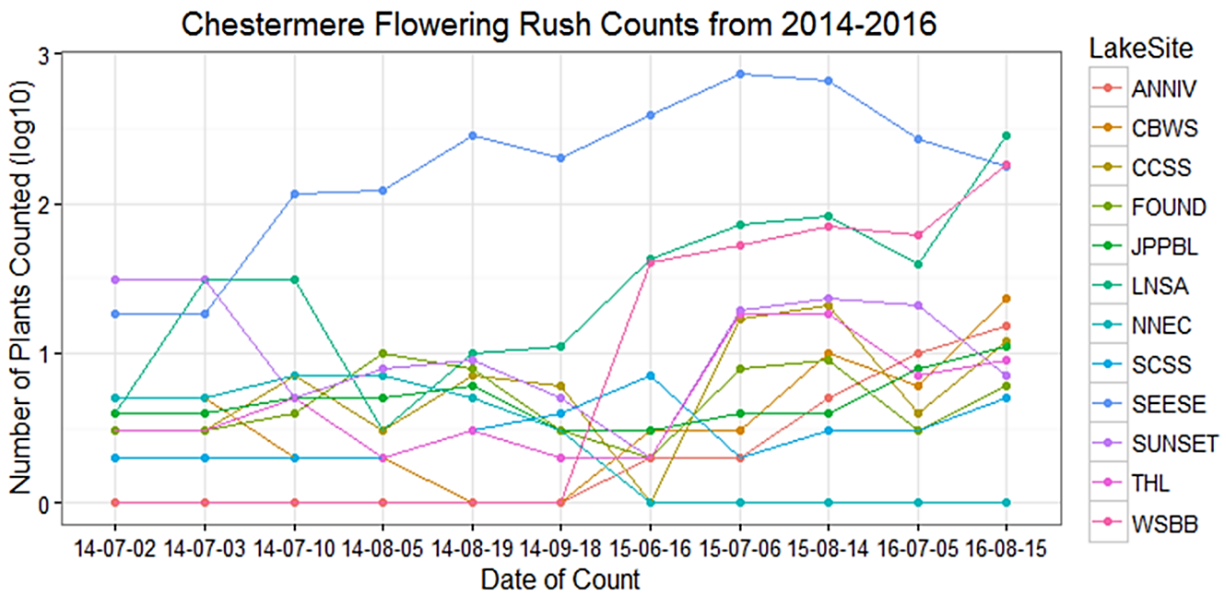
Situated 7km east of Calgary on the Trans Canada Highway is Chestermere Lake, a small man-made irrigation reservoir with an area of 2.65km<sup>2</sup>. Management of the Chestermere irrigation system is by the Western Irrigation District (WID), which supplies irrigation water to over 400 farms and 39 000 hectares of land and delivers municipal water to over 12,000 people in four communities (WID, 2017). Most of the land around Chestermere Lake is privately owned and the lake is popular with residents and visitors for various recreational activities (Mitchell and Prepas, 1990). The lake has a maximum depth of seven meters but is shallow at less than two meters over 50 percent of its area (ALMS, 2015). Appendix B shows the aquatic vegetation I collected in the canal waters in the summer of 2016 and submitted for identification to the University of Calgary Herbarium.

Because of its importance as an irrigation reservoir, the appearance of *B. umbellatus* in the Bow River inflow canal, and in the lake, has raised concerns by the WID over the proper maintenance and delivery of irrigation waters. The seasonal raising and lowering of waters in the reservoir system can create the ideal conditions for spread of *B. umbellatus*, as the warming exposed substrate induces the budding of rhizomes (Hroudova et al. 1996). Thus far, traditional dredging of the middle of the canal, which occurs in the winter, has failed to control *B. umbellatus* along the canal sides but has most likely prevented plants from spreading down into the canal.

Surveys done by the WID in 2014 and 2015 demonstrate the rapid spread of *B. umbellatus* throughout the reservoir (Fig. 1.6). Figure 1.7 shows the increases in the number of plants counted through the summer months from 2014 to 2016.



**Figure 1.6:** Surveys from 2014 (blue points) and 2015 (red points) by the WID showing the rapid spread of *B. umbellatus* in Chestermere Lake. Site of experimental trials is circled (Reprinted with permission from the WID, 2017).



**Figure 1.7:** Number of *B. umbellatus* plants in Chestermere Lake during the growing seasons of 2014 to 2016. Lake sites are as follows: Anniversary, The Cove Beach west shore, Camp Chestermere south shore, Founders, JPP Boat Launch south side, The Landing north shore to Anniversary, North End exit canal, The Sailing Club south shore, South end entrance to South exit, Sunset West side, Town Hall/ Library, West side blue bridge north to residence.

The rapid appearance and spread of *B. umbellatus* in the lake may be the result of improper upstream removal and disposal methods. Large mats of plant material were observed floating down the canal toward the lake in 2014 (Fig.1.8) (M. Quinn, 2017, pers. comm.). Because of the ability of this species to reproduce vegetatively, this plant material would have the opportunity to come to rest near the shoreline and establish new populations.



**Figure 1.8:** Floating mats of *B. umbellatus* that appeared in the inflow canal (a) and later in Chestermere Lake (b) in the summer of 2014. (Photos courtesy of M. Quinn)

### **1.5 Controlling *Butomus umbellatus***

This thesis aims to understand the life history and reproductive characteristics of *B. umbellatus* in Alberta as well as how it responds to various control measures. To help determine effective means of control, the subsequent chapters concentrate on the following objectives:

1. *Characterization of the distribution of ploidy of *B. umbellatus* in Alberta and determination of the effects of ploidy on reproductive investment (Chapter Two).*

The ploidy of a plant can influence its degree of invasiveness through affecting its adaptive ability, via natural selection, and potential to disperse (Lui et al. 2004). Pandit et al. (2011) found that invasive plant species, like *B. umbellatus*, tend to be polyploid in nature or are variable in ploidy

(i.e. having more than one cytotype). Polyploid species may be particularly prone to reallocating resources to vegetative growth, which would benefit the establishment of the plant species in its new environment (Pandit et al. 2011). A plant species may have a different ploidy in an introduced range compared to its native environment, which could change the approach to control methods. As the majority of *B. umbellatus* in Eastern Canada has been classified as diploid (Kliber and Eckert, 2005), and due to its rapid spread in Alberta, I predict *B. umbellatus* in Alberta is diploid because of its ability to reproduce both sexually and vegetatively. As polyploids are often sexually sterile, a diploid's ability to produce seed would provide an effective means of dispersal in a new environment.

2. *Examination of how the degree of investment in rhizomes/bulbils changes the effectiveness of certain control/eradication strategies (Chapter Three).*

In its native range, diploid plants of *B. umbellatus* invest not only in seed production but also in the production of clonal bulbils whereas the triploid variety invests only in the rhizomes and rhizome budding (Hroudova et al. 1996). Because control methods vary in their mode of action on above- versus below-water biomass (e.g., herbicides versus manual removal), the ploidy of *B. umbellatus* will partly determine the effectiveness of these control strategies. It has been noted that in North America, introduced populations of diploid *B. umbellatus* invest more heavily into clonal bulbils than their indigenous counterparts, and flower much more frequently (Brown and Eckert, 2005). An allometric analysis demonstrated that plants from introduced populations of diploids allocated more biomass to clonal and sexual reproduction than indigenous diploids (Brown and Eckert, 2005), which may have allowed introduced populations to rapidly spread in North America.

3. *Performing controlled comparisons of control strategies and determining if replanting of rhizomatous indigenous vegetation increases the effectiveness of eradication methods (Chapter Three).*

Competition between plant species for resources is regarded as a major mechanism responsible for successful invasions (Kohli, 2004, Gloria and Osborne, 2014). Nonindigenous, invasive plants can impact the reproductive success of indigenous plants by changing the movement patterns of pollinators and be a source of foreign pollen to indigenous plants, decreasing indigenous seed set (Da Silva and Sargent, 1996). *B. umbellatus* invades an area more slowly when there is a high density of indigenous vegetation, especially when reed species are present (Hroudova et al. 1996, Madsen et al. 2016). I therefore tested whether the invasion of *B. umbellatus* could be slowed if it encounters intense competition for these resources from indigenous species (Davis et al. 2000).

Based on current knowledge of *B. umbellatus* in Alberta, it is hypothesized that:

1. As vegetative propagation, rather than seed production, will be more important for *B. umbellatus* invasions, especially in deeper water bodies or when in competition with indigenous species, control methods that minimize rhizome/bulbil growth will be most effective;
2. Re-introduction of a rhizomatous indigenous plant will be effective in reducing the ability of *B. umbellatus* to re-establish itself once removed, by introducing a competitor for space and resources.

## Chapter Two:

### **An examination of the reproductive strategies of *Butomus umbellatus* and the determination of ploidy in Alberta**

*Butomus umbellatus* has evolved into two different cytotypes, each with its own unique characteristics. In Europe, part of the native range of *B. umbellatus*, a survey established that 84% of the population was triploid and the remaining 16% diploid (Kliber and Eckert, 2005). In this native range, diploids produce abundant seed and triploids produce little to no viable seed (Lui et al. 2004).

Current evidence suggests the triploid cytotype of *B. umbellatus* is of an autopolyploid origin, occurring from the fusion of a reduced and unreduced gamete (Krahulcova and Jarolimov, 1993). Triploids are sterile with no spontaneous self-pollination or outcross pollination. Any seeds triploids develop to maturity do not germinate. In introduced populations, triploids invest heavily into rhizome biomass rather than into flowers or bulbils (Brown and Eckert, 2005). North American triploids have been previously considered relatively rare (Brown and Eckert, 2005).

The diploid cytotype of *B. umbellatus* is sexually self compatible and can produce up to 20,000 seeds per plant per year. Up to 25% of the plant's biomass can be invested into the production of inflorescences. Another 38% of the biomass of the plant is invested in the production of clonal bulbils (Brown and Eckert, 2005). Bulbils readily detach from the parent plant. They are bouyant and quickly develop on the surface of the water or on moist soil (Fig. 2.1), becoming ramets (clones) of the parent. These small plants can survive and travel on the water's surface until coming into contact with the substrate when they attach and begin new populations.



**Figure 2.1:** A bulbil which detached from the parent plant, floated to the surface of the water and germinated into a new ramet. This ramet will float until encountering the substrate.

With common chromosome counts of either 26 or 39 in the sporophyte (13 in the gametophyte), *B. umbellatus* is documented to have as few as 20 and as many as 40 chromosomes (13-21 in the gametophyte) (IPCN, 2017). There are seven different chromosome counts reported for this species (20, 24, 26, 28, 30, 39, 40), (IPCN, 2017), suggesting that aneuploidy is common. Single chromosome gains and losses are common in plants and these events are usually dysploidy events, which do not alter overall DNA content of cells (Escudero et al. 2014). The impact of aneuploidy on the ecology of plant species is unknown (Segraves, 2017).

In North America, a survey found that *B. umbellatus* had a dramatically higher frequency of diploidy: 71% diploid and the remaining 29% triploid (Kliber and Eckert, 2005). There have been indications of *B. umbellatus* modifying its reproductive strategies since its introduction to North America (Eckert, 2001). North American diploids appear to invest more heavily into bulbil production than their indigenous counterparts (Kliber and Eckert, 2005). The importance of shifts in ploidy in spurring invasions is currently unknown. It is believed that triploid plants were introduced from Europe as horticultural plants but the shift to diploid from triploid occurred after its introduction to North America. As diploids have a greater capacity for clonal reproduction

through bulbils, this could explain the current prevalence of diploids in North America (Kliber and Eckert, 2005).

Diploids can alter their resource allocation without shifts in ploidy. A common observation is that during invasion, normally fertile plants abandon sexual reproduction for clonal reproduction (Eckert, 2001). As clonal offspring tend to be larger, do not require a dormancy period as seeds do, nor require specialized mechanisms of dispersal, clonal reproduction may be favored in a new environment for rapid population establishment (Eckert, 2001). The investment in sexual reproduction is low in *B. umbellatus* in its native range of Europe (Krahulcova and Janolimova, 1993) and in Canada (Brown and Eckert, 2005). Whether the investment in clonal reproduction through inflorescence bulbils, rhizome bulbils, or rhizome buds is altered in a new environment is not known.

During invasion, plant traits that favour rapid multiplication and dispersal may be favored over traditional modes of reproduction in native ranges, thus a species' invasion success depends on its ability to rapidly evolve (Kliber and Eckert, 2005). When reproduction via sexual reproduction is minimal, there may be advantages to being triploid, including the masking of recessive mutations. Duplicated copies of genes may allow the copies to assume new functions that would allow an introduced species to become more plastic and adaptive to its new environment (Madlung, 2012). Polyploidy in plants can potentially change the ecological interactions of the invader with the indigenous population by altering its own phenology (Segraves, 2017). In the case of *B. umbellatus*, no comprehensive study has investigated the role of cytotype variation in invasion success, therefore I sought to characterize the distributions of cytotypes in Alberta. By

learning more about the basic biology of this invasive species in Alberta, we can find effective methods of control of *B. umbellatus*.

If plants in Alberta are found to be diploid, they could be producing seeds and any removal efforts could be replenished by the seed bank. The diversity, continued health and restoration of a wetland depends on its ability to recruit from the seed bank, especially once an invasive plant species is removed (Beas et al. 2013). Heavy infestations of fertile *B. umbellatus* may outnumber other types of seeds present in the seed bank which could potentially hinder a cleared area from recovering itself with indigenous species. Further, if *B. umbellatus* is not producing viable seed, then previous control measures involving the steaming or removal of flower heads will not be effective and resources can be put towards other control strategies. If vegetative propagation is prevalent in *B. umbellatus* invasions, especially in deeper water bodies (when flowering does not occur) or in more competitive settings, control methods that minimize rhizome/bulbil growth should be most effective.

Transitions to triploidy can have numerous effects other than reductions of seeds. Single chromosome gains and losses in polyploids are common and these events are usually dysploidy events that do not alter overall DNA content of cells (Escudero et al. 2014). An increase or decrease in DNA content potentially leads to a change in physiology which may allow a plant to become more stress tolerant to nutrient inputs or water availability, leading to increased competitive ability (Segraves, 2017). Plants that undergo aneuploidy can show a variety of phenotypic changes which can include sterility in normally fertile plants, developmental delays or even changes in plant anatomy (Makarevitch and Harris, 2009, Henry et al. 2010). These

complexities will make the determination of ploidy more difficult and will require the use of a variety of methods to confirm ploidy.

One cellular change that occurs in conjunction with polyploidy, such as the triploid cytotype of *B. umbellatus*, is a larger cell size, including those of the stomata (Madlung, 2013). The aperture size of the stomata is determined by the length of the two guard cells surrounding them which in turn is determined by the size of the genome (Hodgson et al. 2009). The prevailing theory is that increased gene copy numbers seen in polyploids may increase the amount of protein, which in turn increases cell volume (Tsukaya, 2013). In confirming the ploidy of *B. umbellatus*, stomata guard cell length correlates well with the cytotype. Kliber and Eckert (2005), found diploid plants had a mean guard cell length of 40.4 $\mu\text{m}$  ( $\pm 0.9\mu\text{m}$ ) and triploids had a mean guard cell length of 47.2 $\mu\text{m}$  ( $\pm 0.6\mu\text{m}$ ). Stomata guard cell length was not changed by environmental factors and could be used reliably as an indicator of ploidy. To confirm chromosome counts and ploidy, stomata guard cell lengths of plants from the Chestermere canal, Innisfail, Lake Isle, St. Albert and the University of Calgary (U of C) ornamental pond were measured and lengths were correlated with chromosome counts.

Pollen homogeneity also correlates well with ploidy (Lui et al. 2004). The pollen grains from sterile triploid plants are often large and misshapen compared to the fertile pollen from diploid plants which are smaller, round and of a consistent diameter. A previous study found the pollen from diploids ranged in size from 32.3-37.5 $\mu\text{m}$  in diameter and triploid pollen measured 41.1-47.5 $\mu\text{m}$  in diameter (Lui et al. 2004).

By correlating chromosome counts with stomata guard cell lengths and pollen homogeneity, the ploidy of *B. umbellatus* plants in Alberta can be determined with a large degree of confidence.

## **2.1 Determination of ploidy and estimation of *Butomus umbellatus* in the seed bank**

### **2.1.1 Methods**

There are many methods for determining the ploidy of a plant, which range from counting chromosomes visualized with microscopy, to the use of flow cytometry, which measures overall cell DNA content (Doležel et al. 2007). I determined ploidy with a root tip cell squash method. The tips of actively growing roots contain meristematic cells that allow plant roots to continue growing to reach new sources of water and nutrients. Cells in the root meristem are constantly undergoing mitosis, allowing for the visualization of condensed chromosomes, which are normally unseen. A combination of techniques was used to prepare root tips for the visualization of chromosomes based on the work by Mirzaghaderi (2009) and Hiremath and Chinnappa (2015).

Though difficult to obtain exact chromosome counts on all slides, this method was adequate to determine the number of chromosome sets (i.e., with a plant that has either 26 or 39 chromosomes, it is relatively straightforward to estimate ploidy without being able to visualize every chromosome (Chinnappa, pers. comm., 2016)). Because diploid and triploid plants can exhibit aneuploidy within each cytotype (IPCN, 2017), it was important to correlate chromosome counts with stomata guard cell lengths and pollen size and shape to confirm diploidy or triploidy.

To obtain actively growing tissue, plant and rhizome samples of *B. umbellatus* were collected from various locations in Alberta (Table 2).

**Table 2:** Locations, dates, sample form and number of samples collected of *B. umbellatus* for root tip cell squash ploidy testing.

<b>Collection Site</b>	<b>Collection Date (month/year)</b>	<b>Sample Type (Plant or Rhizome)</b>	<b>Number (<i>n</i>) of Samples</b>
Chestermere	06/16	Plant	4
Innisfail	10/16	Rhizome	3
Innisfail	05/17	Rhizome	6
Lake Isle	04/16	Rhizome	6
Sherwood Park	06/16	Plant	1
St. Albert	06/16	Plant	2
U of C	06/16	Plant	2

Plants were collected with roots imbedded in the local substrate to minimize shock to the plant. Plants were replanted at the U of C greenhouse in ordinary top soil and submerged in water. The Chestermere plants were placed outside on the roof of the green house to allow for visitations by pollinators for two months, while the remaining plants were placed indoors in the greenhouse's artificial pond. The rhizomes from Lake Isle were started in pots indoors under natural light and were moved outdoors in June. The October rhizomes from Innisfail were planted in the lab in a combination of top soil and potting soil in a 3:1 mixture and kept submerged in water. The rhizomes from May 2017 were planted outdoors in a container of regular top soil and kept saturated with collected rain water and left for the entire growing season.

To collect root tips, the plants were gently removed from the substrate and rinsed under tap water before 2cm sections of root with intact root caps were cut and placed in 50mL vials of tap

water. To increase the mitotic index of dividing cells for better chromosome visualization, vials of roots were placed on ice for 24 hours (Moh and Alan, 1964). After 24 hours, the root tips were transferred to a fixative solution of three parts 95% ethanol and one-part glacial acetic acid prepared fresh prior to use. For the fixing process, roots were placed in a 1:10 ratio of root material to fixative and were left to fix for 24 hours. After fixing, roots were rinsed in tap water and preserved in a solution of 70% ethanol, also in a 1:10 ratio of roots to ethanol.

To prepare the root tissue for chromosome counting, 1-2 root tips were selected at a time and placed on a microscope slide under a dissection microscope to confirm the tips were intact. If a root cap was present, the roots were placed in a 1M solution of hydrochloric acid and left for 10 minutes to hydrolyze. After softening, the roots were placed on a slide under the dissecting microscope. The root cap was cut off and discarded, and the distal portion of the root was cut to approximately 0.5cm to eliminate as much non-meristematic tissue as possible. One drop of 2% aceto-orcein stain was added to the slide. The root tissue was then macerated with a dissecting needle while on a warming plate to improve stain uptake by the root cells. A coverslip was placed over the tissue and tapped with the back of a pencil to spread the tissue out. Filter paper was placed over the slide and firm downward pressure was applied to the cover slip with the thumb, insuring no lateral movement between the coverslip and the slide. The prepared slide was then examined under a light microscope. Approximately seven to ten squashes were prepared for each plant specimen. Pictures were taken of cells with clear chromosome condensation for later counting. One tailed single sample t-tests (R Core Team, 2016) were performed on the counts to compare chromosome numbers to diploid or triploid plants.

Fresh leaf samples from Innisfail, the Chestermere canal, Lake Isle, St. Albert and the U of C ornamental pond were sent to Dr. John Gaskin of the U.S. Department of Agriculture in Montana to determine ploidy of *B. umbellatus* with flow cytometry. Flow cytometry utilizes optical properties such as fluorescence and the scatter of light to measure microscopic particles in liquid suspension, such as cells and cellular organelles, at a high rate of speed (Doležel et al. 2007). The measure of DNA content is the most popular use of flow cytometry in plant biology. The relationship between ploidy and nuclear DNA content makes flow cytometry suitable for the determination of ploidy level and, under certain conditions, aneuploidy (Doležel et al. 2007). Flow cytometry is not without complications as there may be difficulty in isolating enough intact nuclei for analysis, aggregation of fluorescent particles on the surface of isolated nuclei, or in some cases, cytosolic compounds may interfere with fluorescent staining of the DNA resulting in decreased intensity of fluorescence (Doležel et al. 2007). Flow cytometry was employed to confirm the effectiveness of the cell squash method of ploidy determination.

To prepare samples for examination of stomata guard cells, clear nail polish was applied in a thin layer to two fresh leaves and allowed to dry. A piece of clear acetate tape was placed over the nail polish and smoothed out. The tape was carefully peeled off the leaf, taking a thin layer of leaf epidermis. The tape was placed on a slide and the preparation was examined under a microscope. Forty different stoma were arbitrarily chosen and the guard cells measured. A two-sample t-test (R Core Team, 2016) was performed on guard cell lengths from plants deemed diploid and guard cell lengths from plants deemed triploid to note any significant difference between the two.

To prepare the pollen for examination, mature anthers were collected from two flowers originating from Lake Isle, the Chestermere canal, Sherwood Park and St. Albert. The anthers were placed in clean petri dishes and allowed to dry for 24 hours on the lab counter. Once dried, the anthers were crushed releasing pollen grains. A grain was transferred to a microscope slide and broken up into small pieces and spread evenly on the slide. A drop of Calberla's solution was placed on the sample followed by a coverslip (Galano, 2015). The sample was then placed under a light microscope and the individual pollen grains seen were assessed for size, roundness and similarity between grains. Ten individual pollens were measured for diameter from each plant location.

The extent to which diploid *B. umbellatus* is fertile and populating the seed bank may (1) depend on water depth and (2) affect the amount of long-term monitoring that is necessary post-eradication. I therefore examined variation in the soil seed bank within experimental plots (described further in Chapter Three). Two sediment core samples were taken from each plot, in addition to measuring the specific percentage of *B. umbellatus* coverage by placing a 3 x 3 grid over digital photos of experimental plots. At Lake Isle, plots were categorized for water depth. Plots began inshore in mud, denoted "1", out to a one-meter depth of water which was denoted as "4". Sediment cores were taken using a metal pipe 61cm long with a diameter of 2.54cm. A metal cap was screwed on to one end of the pipe and the pipe was hammered into the substrate to a depth of 20cm (Fig. 2.5). Normal soil seed bank samples are taken to a depth of 5-10cm (Poiani and Johnson, 1988, Yang and Li, 2013). However, due to difficulties caused by this species' ability to form thick mats of rhizomes, it was necessary to sample at a depth of 20cm to penetrate the substrate.



**Figure 2.5:** Sediment core sample collection (left), typical core that was collected (right), in which the first 5 to 7cm was made up of the rhizome layer (arrow).

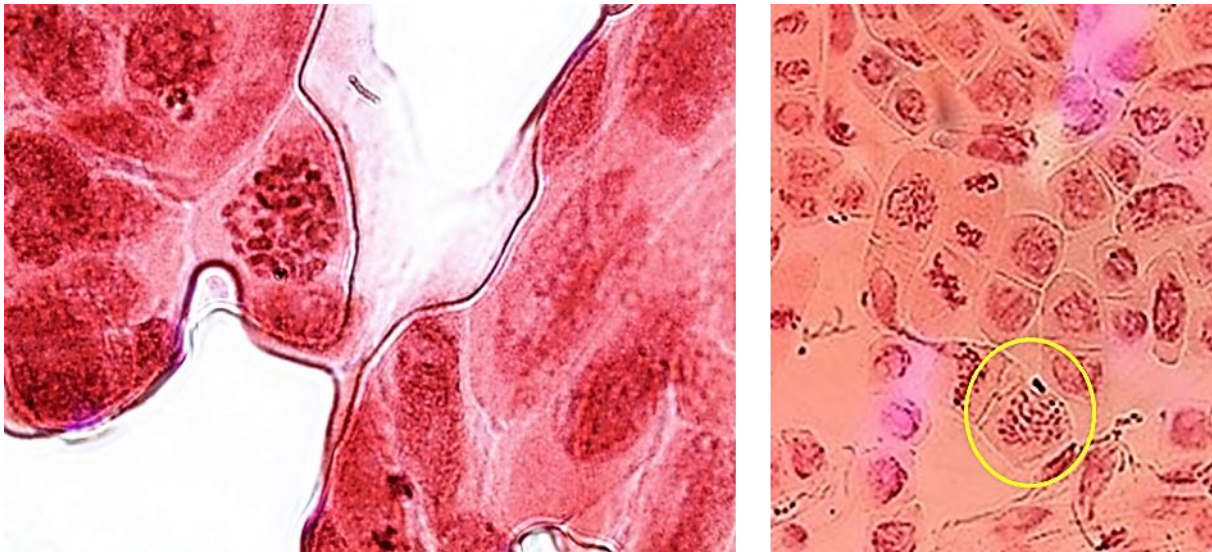
Samples were left to dry on the counter at room temperature for one week. Once dry, the samples were cleaned of plant material and other debris. After mixing the sample, 10g of material was evaluated under a dissecting microscope and intact seeds of all species were removed and counted. A multiple regression analysis (R Core Team, 2016) was performed on the number of seeds in the seed bank as a function of water depth and percent *B. umbellatus* coverage at Lake Isle and the Chestermere canal.

To confirm seed production in diploid *B. umbellatus*, 40 mature, dehiscing seed pods were collected from eight different inflorescence umbels from Lake Isle, the canal at Chestermere Lake and the Sturgeon River in St. Albert. The pods were examined under a dissecting scope and seeds that appeared viable were collected and placed in water in a petri dish. The seeds were placed in a growth chamber at 25°C, with 70% humidity and given 16 hours of light (Hroudová and

Zákravský, 2003) for four weeks to assess if germination, indicated by the appearance of a radicle, would take place.

### 2.1.2 Results

Root tip squashes of cells with condensed chromosomes were distinguishable as large relative to non-dividing cells, with little to no cell wall, allowing a quick assessment of whether the cell preparation was successful. If mitotic cells were observed, I searched for cells with chromosomes condensed and sufficiently separated to allow for counting. Figure 2.2 demonstrates squashes which were performed on roots from plants collected from Lake Isle and Innisfail.



**Figure 2.2:** Root tip cell squashes showing mitotic cells with condensed chromosomes at 1000X magnification. Approximately 26-27 chromosomes ( $2n$ ) can be seen in the cell on the left (Lake Isle) and approximately 30 chromosomes ( $3n$ ) can be seen in the cell on the right (circled) (Innisfail).

The variability in chromosome counts was comparable to that seen by Lui, et al. (2005), who found that populations of diploid plants in Eastern Canada had a mean chromosome count of  $24.7 \pm 1.4$  chromosomes and triploid plants had a mean chromosome count of  $35.6 \pm 2.9$  chromosomes. Alberta diploids had a mean chromosome count of  $24.5 \pm 0.4$  and triploid plants had a mean count of  $30.8 \pm 1.5$  chromosomes.

I observed a wide range in chromosome counts from sampled plants around Alberta, which may be attributed to aneuploidy within plant samples. Results of one-tailed single sample t-tests to determine whether my samples came from a population with either  $2n$  or  $3n$  plants are in Table 3. Chromosome counts were normally distributed as determined for all sample populations except for the St. Albert plants. St. Albert counts could not be transformed so a non-parametric Wilcoxon test was performed. Plants at Chestermere, Lake Isle, Sherwood Park, St. Albert and the U of C ornamental pond are diploid, while the population at Innisfail could be diploid or triploid. Flow cytometry results (Gaskin, pers. comm.) confirmed the plants in Innisfail were triploid, while Chestermere, St. Albert, Lake Isle and the U of C pond plants, though less conclusive, appeared to be diploid. Inconclusive results may be caused by the fibrous nature of the plant material which is preventing the breaking open of enough cells for analysis (Gaskin, pers. comm., 2017).

**Table 3:** Statistical analysis of chromosome counts compared to either a 2n count of 26 chromosomes or a 3n count of 39 chromosomes based on one tailed single sample t tests, achieved via root tip cell squashes with “*n*” denoting the number of counts performed.

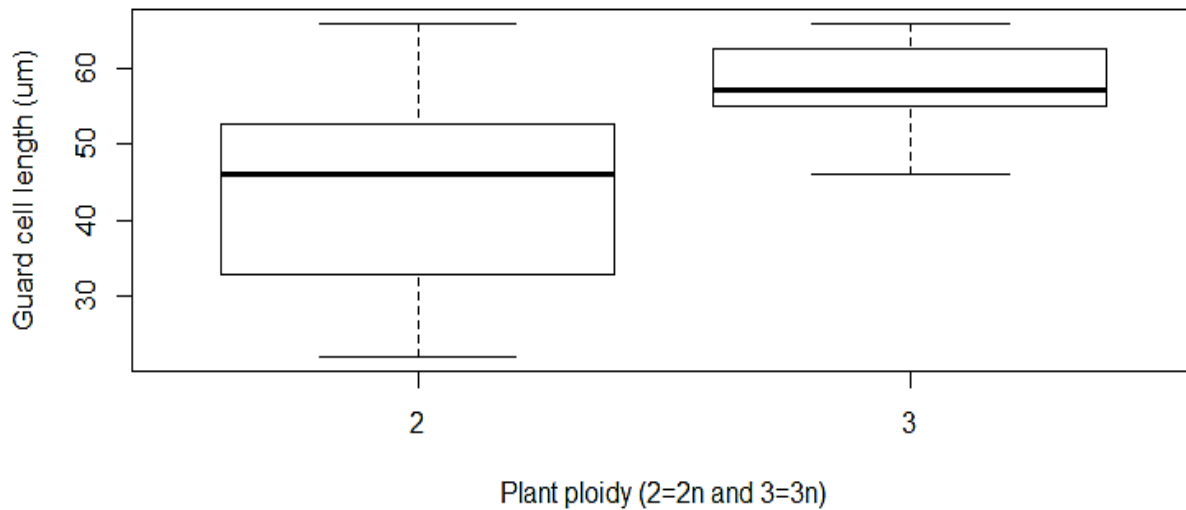
<b>Location</b>	<b><i>n</i></b>	<b>Mean ± SE</b>	<b>Ploidy</b>	<b>P value</b>	<b>Ploidy</b>	<b>P value</b>
Chestermere	9	22.1 ± 1.16	2n	=0.994	3n	=8.47e-07
Innisfail	27	30.8 ± 1.55	2n	=0.005	3n	=0.0001
Lake Isle	12	24.7 ± 0.45	2n	=0.995	3n	< 2.2e-16
Sherwood Park	3	26.0 ± 3.06	2n	=0.500	3n	=0.026
St. Albert*	14	22.4 ± 0.80	2n	=0.994	3n	=5.15e-4
U of C pond	20	24.5 ± 0.67	2n	=0.984	3n	=3.25e-15

\*Wilcoxon non-parametric test

Guard cell measurements showed marked differences associated with ploidy (Table 4 and Fig. 2.3). Statistically, measurements did not display normality and could not be transformed so a non- parametric Wilcoxon test (R Core Team, 2016) was used to compare diploid to triploid mean cell length. Triploid plants had a significantly larger mean guard cell length than diploids (Mean ± SE: Triploids = 58.02µm ± 0.79µm; Diploids = 43.44µm ± 0.91µm;  $W = 626, p < 0.0001$ ) which is similar to a previous study by Kliber and Eckert (2005) (Fig 2.3).

**Table 4:** Stomata guard cell measurements (in micrometers) of *B. umbellatus* leaves from the U of C pond, Chestermere, St. Albert, Lake Isle and Innisfail. Means are from measurements of 40 guard cells.

Length ( $\mu\text{m}$ )	Chestermere	Innisfail	Lake Isle	St. Albert	U of C Pond
Minimum	44.0	46.2	33.0	22.0	41.8
Maximum	57.2	66.0	66.0	33.0	52.8
Mean	51.7	58.1	49.9	26.2	46.0



**Figure 2.3:** A box plot comparison between guard cell length and inferred plant ploidy. Triploid plants had a mean guard cell length of  $58.0\mu\text{m} \pm 0.8\mu\text{m}$  and diploid guard cell lengths measured  $43.4\mu\text{m} \pm 0.9\mu\text{m}$ .

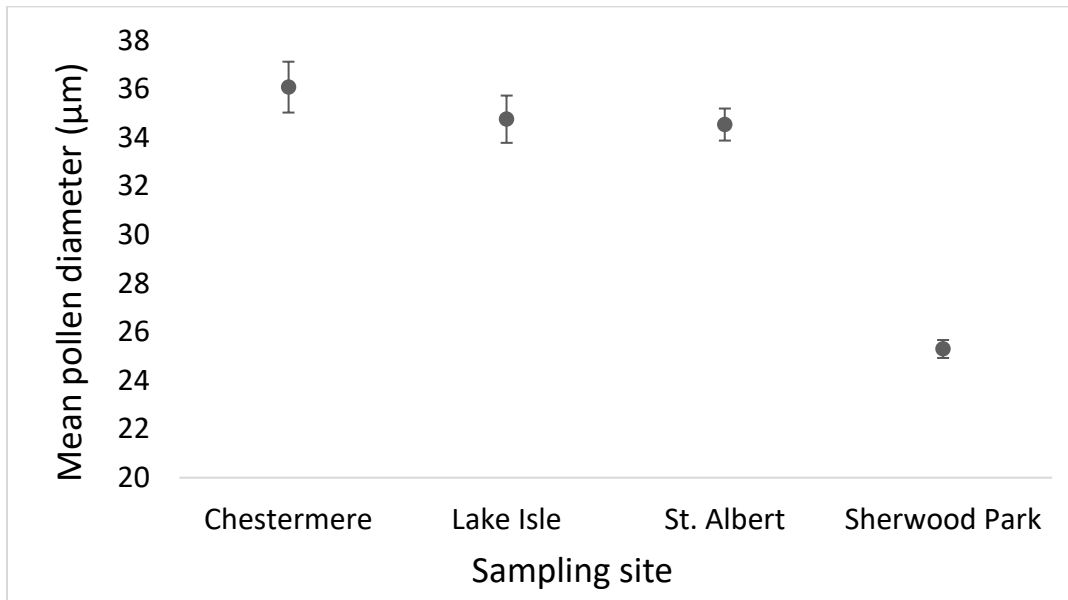
Pollen appeared to be uniform in diameter and roundness for diploid plants. The pollen sample from Sherwood Park was the smallest in average pollen diameter, but within samples,

pollen diameter exhibited little variation in diameter (Fig. 2.4), having an average standard error of  $\pm 0.76\mu\text{m}$  over 10 measurements (Table 5).

**Table 5:** Mean ( $\pm$  SE) pollen diameter of 10 pollen grains. Results comparable to Lui et al. (2004), where diploids ranged in size from 32.3-37.5 $\mu\text{m}$  in diameter and triploid pollen measured 41.1-47.5 $\mu\text{m}$  in diameter.

<b>Sample Location</b>	<b>Average Pollen Diameter (<math>\mu\text{m}</math>)</b>	<b><math>\pm</math> SE (<math>\mu\text{m}</math>) <i>n</i>=10</b>	<b>Inferred Plant Ploidy</b>
Chestermere	36.1	1.04	2n
Lake Isle	34.8	0.97	2n
Sherwood Pk.	25.3	0.37	2n
St. Albert	34.5	0.66	2n
Innisfail	N/A	N/A	3n

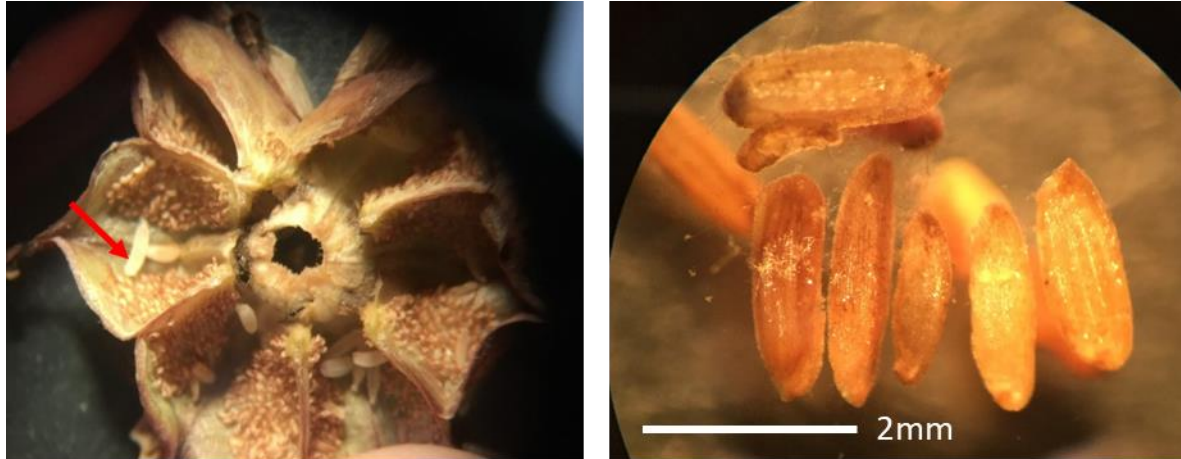
In North America, triploid plants rarely flower under field or green house conditions (Kliber and Eckert, 2005). Despite several attempts, collected samples from the Innisfail triploid population did not flower. Plants on site in Innisfail have been sprayed with herbicide over several seasons so no flowers were seen and therefore pollen could not be collected for examination.



**Figure 2.4:** Mean pollen diameter over 10 measurements  $\pm$  SE from inferred diploid pollen from various locations in Alberta.

Percent coverage of experimental plots by *B. umbellatus* at Lake Isle was calculated to be 70-100% and 15-67% coverage at the Chestermere canal. No *B. umbellatus* seed was seen in any of the sediment samples taken from Chestermere or Lake Isle. As the canal at Chestermere Lake is kept unvegetated, there was a very low number of non- *B. umbellatus* seeds observed (0-2 seeds per 10g of soil). The total number of seeds (belonging to all species) was not associated with water depth at Lake Isle or percent coverage of *B. umbellatus* ( $F_{2,16} = 0.44$ ,  $p > 0.05$ , water depth:  $t = -0.69$ ,  $p > 0.05$ , coverage:  $t = 0.84$ ,  $p > 0.05$ ). My examination of ripe seed pods revealed that out of the hundreds of ovules that could potentially become seeds, most ovules did not mature or aborted early in development (Fig. 2.6). Out of 30 seeds collected from Chestermere Lake, 16

seeds from Lake Isle and 14 seeds from St. Albert that appeared viable (Fig. 2.6), no germination was seen in any of the collected seeds.



**Figure 2.6:** (Left) An example of a mature seed pod from *B. umbellatus*. Note how few ovules have matured into something resembling a seed (arrow). (Right) Seeds collected for a germination test.

### 2.1.3 Discussion

With five *B. umbellatus* populations sampled, Alberta diploids had a mean chromosome count of  $24.5 \pm 0.4$  and triploids a mean count of  $30.8 \pm 1.5$ . These values were not close to the expected values of 26 or 39 for diploids and triploids respectively. It is possible that lower chromosome counts for diploids may be attributed to not visualizing every chromosome due to stacking rather than chromosomes not being present. Higher counts may be due to chromosome fragmentation (Ahloowalia, 1965). Aneuploidy may also play a role in these unexpected counts. Chromosome counts by Lui et al. (2005) also yielded mean counts smaller than expected values. Speculation was that smaller chromosomes are more difficult to visualize; therefore, it was important to correlate counts with guard cell lengths and pollen size.

Through determining chromosome counts and correlating counts with stomata guard cell lengths and pollen homogeneity, I infer *B. umbellatus* in Alberta to be primarily diploid with the exception of a triploid population in the Innisfail area. More widespread testing should be done to confirm these findings, as only a small number of samples from each population were tested.

Hroudová and Zákřavský (2003) found a greater percentage of *B. umbellatus* seeds germinated after overwintering outdoors under water or buried in 1-2cm of sediment. While stratification might have improved the germination rate of seeds, sexual reproduction was considered too low to be a substantial contribution to population growth for *B. umbellatus*. Diploid *B. umbellatus* may not be producing seed as rhizomatous plants will invest more into vegetative propagation when not crowded by other plants, insuring spread of the genet (Armstrong, 1982, Winner et al. 2012). Clonal growth is also advantageous when horizontal spread is favoured (Bazzaz et al. 1987). Plant species that span a range of latitudes or altitudes may increase vegetative reproduction over sexual (seed) reproduction with a decrease in the length of the growing season (Winner et al. 2012). As *B. umbellatus* is still a relative newcomer to Alberta, this may explain why diploid plants are not producing seed despite flowering. Fernando and Cass (1997) suggested that a long lived clonal plant like *B. umbellatus* may accumulate deleterious genetic mutations which may contribute to developmental irregularities during gamete formation causing sterility of gametes.

The lack of seeds in the seed bank, an apparent lack of seed production in mature seed pods and the failure of seeds to germinate, would suggest that the diploid cytotype of *B. umbellatus* is not investing heavily into sexual reproduction in Alberta. These results are consistent with the findings of Brown and Eckert (2005) and indicate control measures should be directed towards

vegetative reproduction and growth. As the number of non- *B. umbellatus* seeds was not affected by the percent coverage of *B. umbellatus* this would seem to indicate the possibility of natural regeneration of an area once *B. umbellatus* has been removed.

### Chapter Three:

#### ***Butomus umbellatus* control trials at Lake Isle and the Chestermere inflow canal**

*Butomus umbellatus* can spread rapidly throughout an aquatic system, outcompeting indigenous vegetation and dramatically transforming the ecosystem. Given the large potential economic impacts to both the irrigation and leisure industries in Alberta, it is advisable to find effective ways to control or eradicate populations of *B. umbellatus*. Current control methods, including clipping flower heads, steaming the vegetation, and performing cutbacks of the leaves, have appeared ineffective. While these methods are inexpensive, they neglect the large contribution of clonal reproduction to population growth in this species. More effective strategies for a clonal species may include:

1. Benthic barriers: Benthic barriers (BB) involve installing a dark fabric covering over populations of *B. umbellatus*. If the plants are covered and unable to photosynthesize, plants are forced to tap into existing carbohydrate reserves. With depleted reserves, the plants may become less viable. In a plant that relies on clonal propagation, which is normally built from acquired or stored resources, preventing carbon fixation via photosynthesis can cause plants to defer reproduction for survival (Winner et al. 2012) making the spread of plants via bulbil production less possible.

2. Indigenous plant restoration: In other invaded systems, replanting of indigenous vegetation (RP) reduces the abundance of nonindigenous species through competition (Brown et al. 2008). Re-establishing an indigenous population of plants through restoration, once *B. umbellatus* has been removed from an area, potentially impedes the reestablishment of *B. umbellatus*. At Lake Isle, it was observed that *B. umbellatus* colonized areas cleared by property

owners more quickly than areas that had abundant indigenous vegetation. It was observed that *B. umbellatus* was suppressed by the presence of reed-bed species in a native range (Hroudova et al 1996). By revegetating an area cleared of *B. umbellatus* with indigenous rhizomatous plant species, it may be possible to prevent or slow the reestablishment of *B. umbellatus*.

3. Manual removal: A complete manual removal (MR) of *B. umbellatus* from plots can be attempted to insure no bulbils or rhizomes remain. As bulbil production is higher in North American diploids (Brown and Eckert, 2005) and rhizome fragments as small as 1cm in length have been observed to germinate, it is vital to insure all vegetative material has been removed from the substrate (Berger, 1993), otherwise reestablishment of the *B. umbellatus* population can occur. Manual removal requires careful attention, as it is necessary to insure buoyant bulbils or rhizome fragments do not escape and populate new areas.

### **3.1 Lake Isle and Lake Chestermere *B. umbellatus* control trials**

In the spring and summer of 2016, trials with each of the above treatments were initiated to determine the most effective means of control in areas of invasion by *B. umbellatus*. The primary site of research was within the boundaries of Camp Koininia, on the north shore of the western basin of Lake Isle. The invasion by *B. umbellatus* has been progressing for many years and in 2007, it was documented as a serious problem to the ecology of the area and for recreational users of the lake (D. Buchholtz, 2016, pers. comm.). Appendix C provides a copy of a letter submitted by Doug Buchholtz who grew up at Lake Isle and his observations on the spread of *B. umbellatus* at the lake. The camp has been trying to combat the problem by cutting plants below the water line each year. A mechanical harvester was used in deeper waters which also cuts plants below the water line. While not part of my proposed research, I was able to provide an observational

analysis on the effects of long term cutbacks on the plants (detailed in Appendix D). Alberta Environment and Parks is investigating more rapid forms of control and attempted an herbicide trial within the boundaries of the camp. The permitting of herbicides for aquatic use is highly regulated and can result in delays that reduce the effectiveness of treatments. While I removed this experimental treatment from my research design due to concerns regarding its feasibility, I have provided a summary of preliminary results (see Appendix D).

Given the difficulties and risks involved in herbicide applications, I conducted herbicide-free controlled trials to compare different treatment methods and efficacy in reducing *B. umbellatus* biomass. Measuring reductions of an invasive plant between growing seasons often involves comparing baseline levels of the invasive species to those after a control measure was applied (Before–After–Control–Impact (BACI) design (Murtaugh, 2002)). Rather than focus solely on abundance or density of *B. umbellatus*, I was also interested in how rhizome biomass (a critical determinant of spread) of *B. umbellatus* was affected by the treatments. Because *B. umbellatus* is a patchily distributed species, variation in density can influence the amount of carbohydrate reserves in the rhizomes for regeneration the following year. Increased density (i.e. percent coverage within designated plots) has been seen to decrease rhizome size and biomass (Hroudova et al. 1996). Similarly, water depth influences the proportion of resources invested in stem biomass and may also be associated with decreased rhizome biomass (Grace, 1989). At Lake Isle, *B. umbellatus* is not uniformly distributed through the experimental sites, therefore, I took a series of pre-treatment plant samples in 2016 to measure its current abundance and biomass. I wanted to examine if percent coverage was a factor influencing plant growth and rhizome size (i.e., if dense patches of *B. umbellatus* had smaller rhizomes). Likewise, water depth is not uniform

throughout the sites, so I also determined whether depth of water impacted underground rhizome/bulbil biomass production, rhizome size or percent coverage of *B. umbellatus*.

I also examined the effects of cutbacks on rhizome size and biomass production (Appendix D). Hroudova et al. (1996) found if the plants are cut back, they are unable to photosynthesize and must rely on carbohydrate reserves in the rhizomes to maintain growth. The end result is a decrease in rhizome size and possibly biomass as well.

### **3.2 Methods**

Before commencing control trials, pre-treatment plant samples were taken to obtain baseline measurements of *B. umbellatus* which included underground rhizome/bulbil biomass production (henceforth referred to as biomass or dry mass), rhizome circumference (size) and percent coverage of rush. Initial tests allowed me to determine whether environmental variations within my study sites were strong enough to influence the effects of my experimental control treatments.

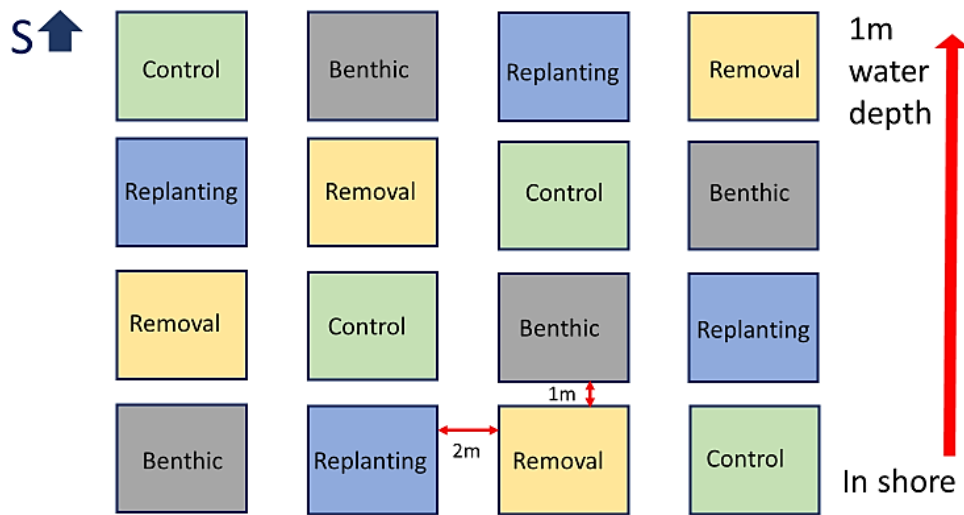
The Chestermere canal presents a very different environment to that of Lake Isle. Plants exhibited delayed maturity compared to Lake Isle and at the start of June 2016, were just emerging from the water. Water depth was not considered a variable due to the steep slope of the sides of the canal; therefore, only percent plant coverage was examined. Replanting trials with indigenous vegetation was not performed due to the canal's unvegetated state. An area of trial suction harvesting of plants took place at the same time as the control trials and observations on the effects of this treatment can be found in Appendix D.

### 3.2.2 Lake Isle

A series of 2m x 2m plots were established in June 2016 to the east of the beach and boat launch areas of the camp, beginning inshore and moving out to a water depth of 1m. (Geographical Coordinates: 53.5997, -114.8008) (Fig. 3.1). Plots were separated east-west by 2m and north-south by 1m (Fig 3.2). The 4m<sup>2</sup> plots were chosen as they allowed reaching into the plot to take samples without having to disturb the rhizome bed and potentially changing the measured variables. Using a Latin square configuration, four control plots, four MR plots, four BB plots, and four RP plots were established (Fig. 3.1). Digital photos were taken of each plot for determination of *B. umbellatus* coverage within the plots by placing a 3 x 3 grid over each picture and calculating the percent coverage by *B. umbellatus*.



**Figure 3.1:** Overhead view showing the location of experimental plots on the north shore of Lake Isle at Lat. 53.5997, Long. -114.8008. (Imagery©2017 DigitalGlobe, Map Data©2017 Google).



**Figure 3.2:** Establishment of experimental plots on the north shore of Lake Isle using a Latin square.

Sediment samples were collected from each plot, (See Chapter Two), and for consistency, the samples were taken from the northeast and southwest corners of each plot. At this time, a pre treatment plant sample was also collected, one from the east and one from the west side of each plot.

As *B. umbellatus* rhizomes form an entangled mat 7-10cm in depth, it was not possible to calculate rhizome investment per individual plant. To determine rhizome investment and biomass production, a sample was obtained from the total material that came up with a 15cm x 15cm spade. The spade was fully inserted into the substrate to a depth of 15cm to obtain a standardized volume of sediment and rhizomes. For uniformity, the leaves of pre treatment plant samples were cut back to a length of 30cm, and the plant was carefully washed in the lake water to remove excess substrate before it was placed in a labelled bag. Post treatment plant samples would be taken the following

growing season using similar sampling methods. Plots were sampled from the north and south ends in 2017 to ensure there was no influence from the removal of samples in 2016.

Upon return to the University of Calgary, collected plants were transferred to paper bags and placed into a drying chamber at 32°C and low humidity where they were allowed to dry for one week to achieve a dessicated state before being weighed. Five different rhizome fragments were arbitrarily collected from each sample and their circumferences measured. Pre treatment samples collected were given a water depth designation of 1-4, “1” denoting shoreline and “4” at 1m of water (as in Chapter Two). A multiple regression analysis (R Core Team, 2016) was then performed to address whether: (1) water depth determined biomass, rhizome size, or plant coverage; (2) the percent coverage of *B. umbellatus* determined biomass or rhizome size as previously discussed in section 3.1.

The three eradication treatments described above were initiated in July 2016. Cleared, plots were checked by hand to insure rhizome fragments had not been left behind in the sediment. Four cleared plots were then replanted with indigenous, local *Schoenoplectus tabernaemontani* (softstem bulrush) or *Typha latifolia* (cattails) transplanted from other areas of the shoreline. These plants were planted at 1 plant/50cm within the 4m<sup>2</sup> plot with two plots containing cattails and two plots containing bulrush.

For the placement of benthic barriers, *B. umbellatus* was cut back to approximately 30cm prior to barrier placement. Once trimmed, 2m x 2m wooden frames holding a geotextile fabric (landscape fabric which prevents sunlight from penetrating and allows the escape of gas) were put into position. In deeper water, these frames were weighed down at the corners with stones or

blocks of cement. Barriers were kept in place for 13 weeks and then removed in October prior to icing of the lake.

Owing to impending widespread herbicide spraying scheduled to occur at the beginning of July 2017, post treatment samples were collected at an earlier date than in 2016. To determine if there was a difference in sampling time and technique between 2016 and 2017 treatment seasons, control plots were evaluated using paired t-tests to determine any significant differences between the growing seasons.

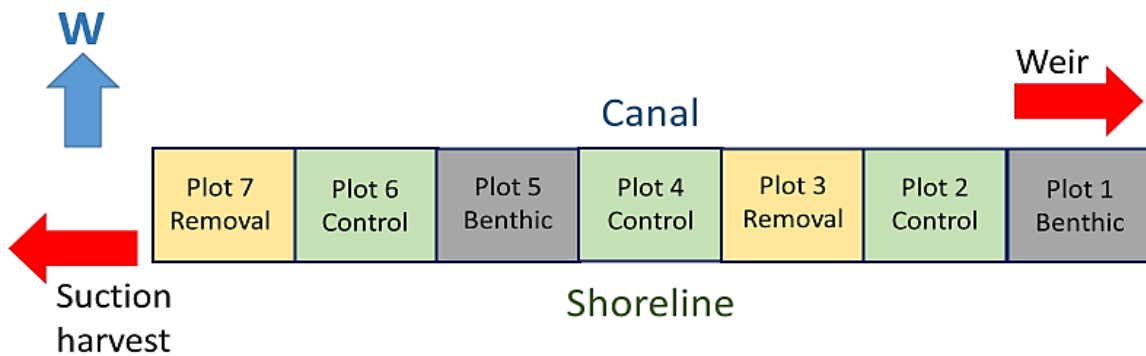
### **3.2.2 Chestermere Lake**

Differences in landscape, use and resources necessitated some modifications to the experimental design used at Chestermere Lake. In July 2016, experimental plots were established and samples collected from the inflow canal of Chestermere Lake at Drop Point 1 (Geographical Coordinates: 51.0150, -113.8433), upstream of a weir (Fig. 3.3). Seven 2m x 2m plots were established from the shore margin. Plots were confined along the edge of the canal due to the steep grade of the canal sides.

Treatment plots were alternated side by side with control plots on the west bank of the canal (Fig. 3.4). Digital photos were taken of each plot for coverage estimates in each growing season.



**Figure 3.3** Overhead view of location of experimental plots at the inflow canal of Chestermere Lake at Lat. 51.0150, Long. -113.8433. The star indicates the southern end of Chestermere Lake. (Imagery©2017 DigitalGlobe, Map Data©2017 Google)



**Figure 3.4:** Configuration of plots along the Chestermere inflow canal. To the west is the canal and to the east is the shoreline. All plots were in a consistent depth of water.

For pre-treatment sampling, sediment core samples were taken from the south side of each plot for soil seed bank testing (as described in Chapter Two). Pre-treatment plant samples for

biomass and rhizome circumference measurements were taken from the south side of the plots. Post treatment samples the following growing season were taken from the north side of each plot. Plant and soil samples were processed as previously described and percent coverage of plots by *B. umbellatus* was calculated. BB placement and MR treatments were carried out like Lake Isle, with the BBs left in place for 14 weeks.

Consistent with a BACI design, I examined between-year differences. This was a smaller scaled experiment lacking in power for a true BACI design (Murtaugh, 2002), due to changes measured over only one season which had the potential to be small in nature, thus reducing the power (Schwarz, 2015). Effectiveness of treatments between years (measured as the difference between 2017 and 2016 rhizome biomass, size and percent coverage from regrowth), was analyzed as a two-factor nested ANOVA with pairwise comparisons between treatments (R Core Team, 2016). This was chosen as there are two nominal variables to consider: lake site and treatment type. The treatments and effects were monitored for only one season and there were few replicates due to complications that will be discussed later. Murtaugh (2002) suggested temporal differences between test periods, such as year to year temperature or rain fluctuations, can limit the power of a BACI design. Murtaugh suggested analysis of before and after studies is better served using graphical displays, knowledge of the studied system and common sense rather than relying solely on p-values. To this effect, I have included graphical displays of pre-and post treatment measurements in the next section.

### 3.3 Results

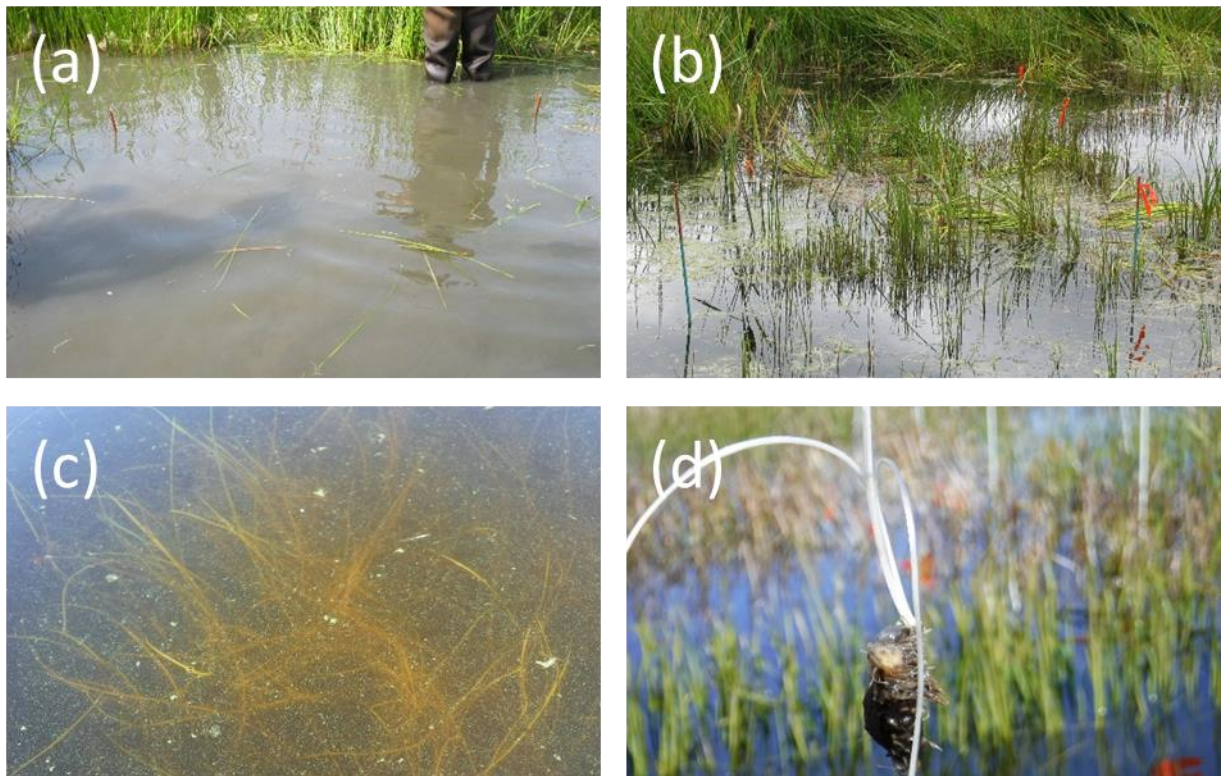
After a  $\log_{10}$  transformation of drymass data for normality, regression analyses of Lake Isle data revealed no associations between rhizome size, water depth, or percent coverage ( $F_{2,16} = 1.45$ ,  $p > 0.05$ ), with both predictor variables having a non-significant effect. There was also no association between biomass, water depth or plant coverage ( $F_{2,16} = 1.05$ ,  $p > 0.05$ ). Similar to Lake Isle, Chestermere Lake pre-treatment analysis of plant coverage had no significant effect on rhizome circumference ( $F_{1,5} = 4.42$ ,  $p > 0.05$ ) nor on plant biomass ( $F_{1,5} = 1.30$ ,  $p > 0.05$ ).

#### 3.3.1 Lake Isle

It should be noted that the spring of 2016 was unseasonably warm and spring growth was a month ahead of schedule. Flowering Rush was apparent by the end of April, which was considered very early. By the time experimental plots were set up in June, *B. umbellatus* was  $\geq 1\text{m}$  high and flowering.

Considerable amounts of biomass were removed from the MR and RP plots at Lake Isle with an estimated 400 to 600 plants taken from each 2m x 2m area. The rhizome bed was 7-10cm in depth. MR plots were observed four weeks later and substantial regrowth had occurred in these plots (Fig.3.5a-b).

Benthic barriers were removed after 13 weeks. Plants under the barriers were ribbon like and chlorotic (lacking green chlorophyll) (Fig. 3.5c-d). The RP plots were assessed in October and replanted cattails and bulrush were present and growing.



**Figure 3.5:** (a) A newly cleared plot of *B. umbellatus* and (b) the same plot 4 weeks later, showing rapid regrowth, (c) ribbon like and (d) chlorotic appearance of *B. umbellatus* after benthic barrier removal.

Upon revisiting plots the following year (June 2017), the water was at its highest level in 25 years (as reported by the camp residents). Plots that were on the shoreline in 2016 were now submerged under one meter of water. The changing water levels likely affected the replanting treatment: the two cattail replanted plots had only 1-2 cattails and none of the replanted bulrushes were observed. A further complication of 2017 sampling was the explosion of growth of other aquatic weeds (See Appendix A). The mats of weeds were extremely thick and covering all experimental plots, making sampling difficult. Due to the deep water and thick weeds, only 10 of

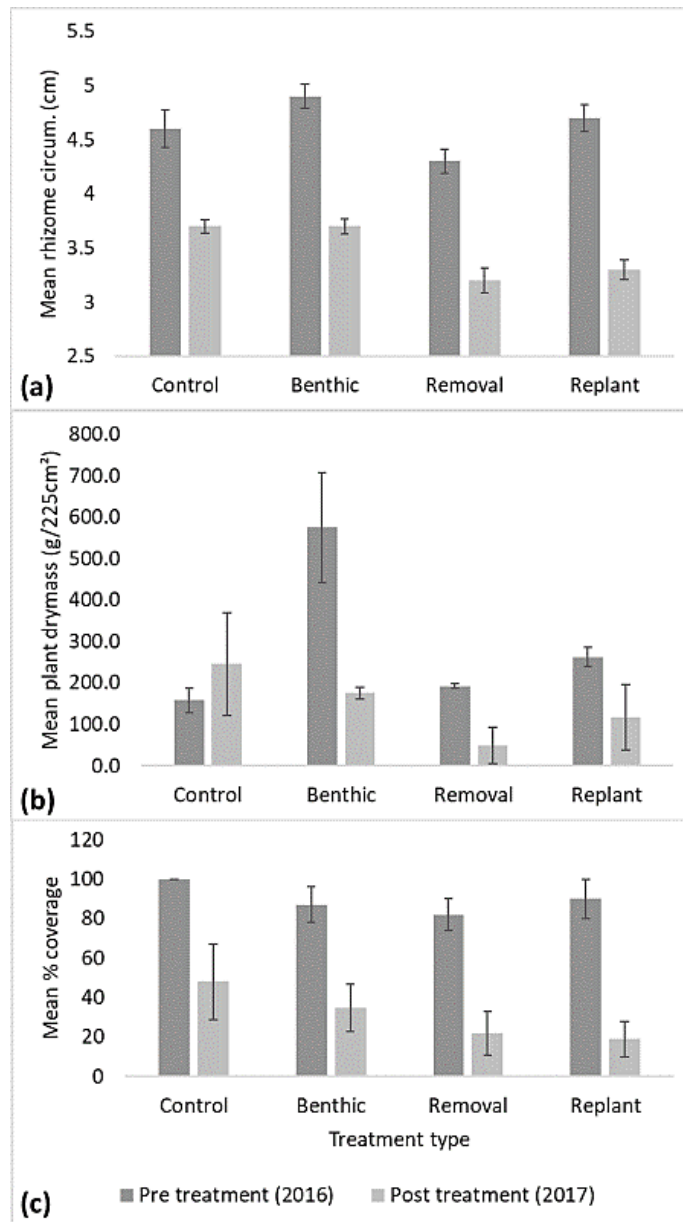
the 16 plots could be resampled for post treatment measurements: three controls, three benthic, two removals and two replanted plots. Digital photos were taken for estimates of percent coverage by *B. umbellatus*.

For the control plots, rhizome size did not significantly differ between years, nor did biomass. Percent coverage of control plots between seasons also did not significantly differ (Table 6)

**Table 6:** Comparison of control plots at Lake Isle between 2016 and 2017 for the variables of rhizome size, biomass and percent coverage of plots by *B. umbellatus*.

	<b>2016</b>	<b>2017</b>	<b>t statistic</b>
Mean rhizome size ±SE (cm)	4.6 ± 0.4	3.7 ± 0.1	$t_2 = 2.4, p > 0.05$
Mean biomass ±SE (sqrt(g/225cm <sup>2</sup> ))	157.6 ± 30.4	245.2 ± 123.6	$t_2 = -0.6, p > 0.05$
Percent coverage ±SE	100	48.3 ± 18.7	$t_2 = 2.8, p > 0.05$

The average rhizome size and biomass per plot ± SE for each treatment type between the 2016 and 2017 growing seasons are shown in Figure 3.6a-b. Rhizome circumference decreased slightly among all plots by the same extent between growing seasons. The benthic barrier plots displayed the greatest decrease in biomass. Percent coverage of *B. umbellatus* decreased for all plots in the second year. Biomass was square root transformed and, using a two-factor ANOVA (R Core Team, 2016), there was a significant difference in mean rhizome size, biomass and percent coverage between 2016 and 2017 over all plots (Table 7).



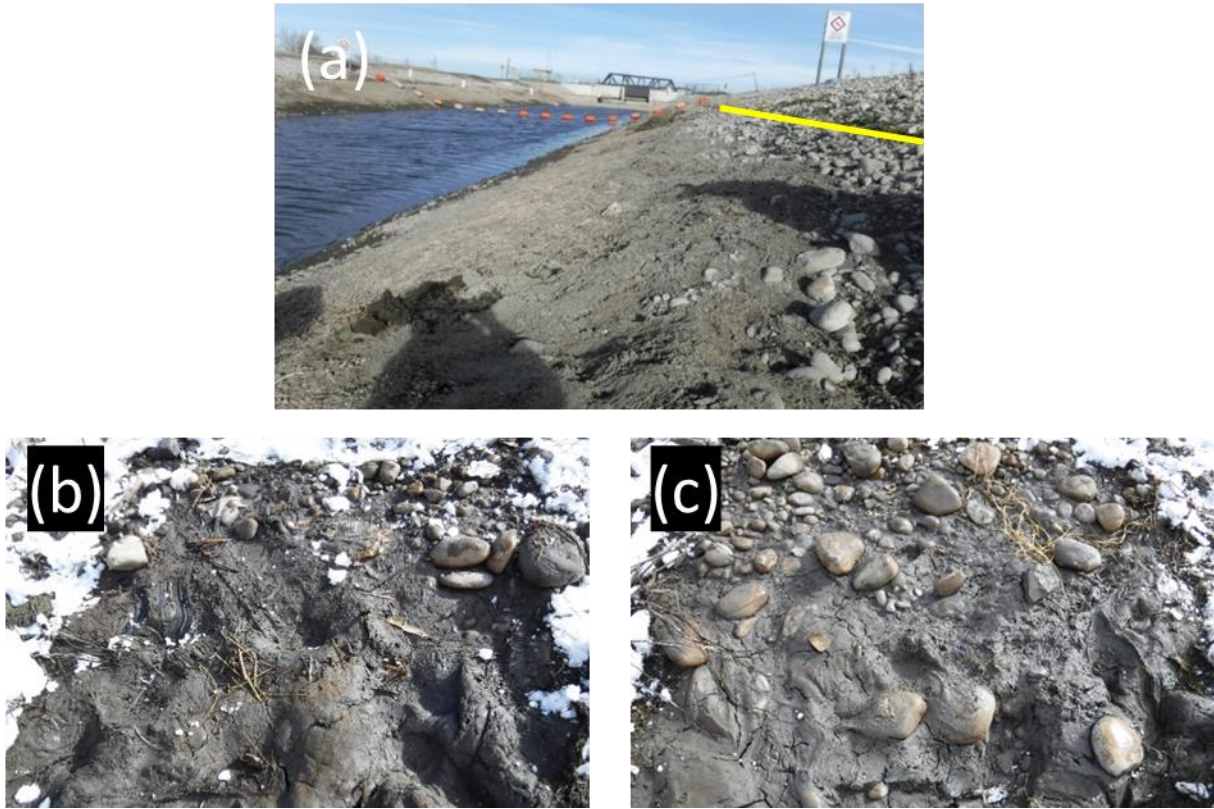
**Figure 3.6:** For treatments at Lake Isle, (a) average rhizome size (cm) over 5 measurements  $\pm$  SE of each combined treatment regime before (2016) and after (2017) treatments, (b) average biomass (g/225cm<sup>2</sup>)  $\pm$  SE over 3 plots each of control and BB and 2 plots each of the MR and RP., (c) average *B. umbellatus* percent coverage estimates over 3 plots each of control and BB and 2 plots of MR and RP  $\pm$  SE.

**Table 7:** Comparison of all 10 experimental plots at Lake Isle between 2016 and 2017 for the variables of rhizome size, biomass and percent coverage of plots by *B. umbellatus*.

	2016	2017	ANOVA
Mean rhizome size ±SE (cm)	4.6 ± 0.1	3.5 ± 0.1	$F_{3,15}=60.0, p < 0.0001$
Mean biomass ±SE (sqrt(g/225cm <sup>2</sup> ))	310.7 ± 68.7	159.2 ± 42.2	$F_{3,15} = 5.8, p < 0.05$
Mean percent coverage ±SE	90.5 ± 3.7	33.3 ± 7.2	$F_{3,15} = 55.5, p < 0.0001$

### 3.3.2 Chestermere Lake

Plants at Chestermere Lake experienced two very different growing seasons from 2016-2017 as the spring of 2017 was cooler than the spring of 2016. During the summer, MR plots were visually assessed, and no regrowth of *B. umbellatus* was seen by the end of the 2016 growing season. By October, the canal water level was lowered, and all plots were out of the water (Fig. 3.7a). The benthic barriers were removed after 14 weeks and appeared to have been effective in dampening the growth of *B. umbellatus* (Fig 3.7b-c).



**Figure 3.7:** (a) Chestermere inflow canal plot area after the water level had been lowered. Original shoreline is shown with the line. (b-c) Benthic barrier plots in October 2016 after 14 weeks of the barriers in place. There is little evidence of *B. umbellatus* present.

Using paired t-tests, the three control plots exhibited no significant difference in rhizome circumference nor was there a significant difference in biomass between sample years. Percent coverage of *B. umbellatus* growth in each control plot did not significantly differ between growing seasons (Table 8).

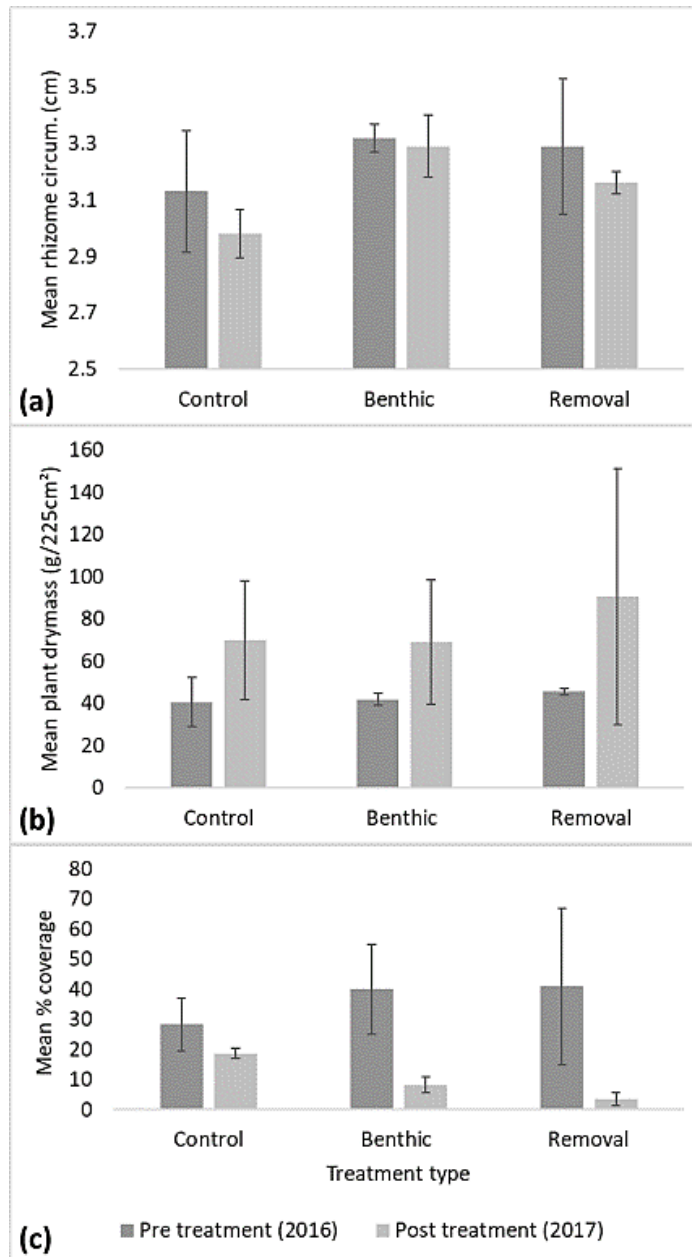
**Table 8:** Comparison of control plots at the Chestermere inflow canal between 2016 and 2017 for the variables of rhizome size, biomass and percent coverage of plots by *B. umbellatus*.

	<b>2016</b>	<b>2017</b>	<b>t statistic</b>
Mean rhizome size ±SE (cm)	3.1 ± 0.2	3.0 ± 0.1	$t_2 = 1.0, p > 0.05$
Mean biomass ±SE (g/225cm <sup>2</sup> )	40.6 ± 11.5	69.9 ± 28.1	$t_2 = -1.6, p > 0.05$
Mean percent coverage ±SE	28.3 ± 8.8	18.7 ± 1.7	$t_2 = 1.3, p > 0.05$

All biomass data was  $\log_{10}$  transformed for normality and equal variance. Comparing each treatment regime, rhizome size did not decrease significantly, and biomass increased between years. Percent coverage decreased significantly between the two growing seasons with the BB plots showing the greatest difference (Table 9 and Fig. 3.8).

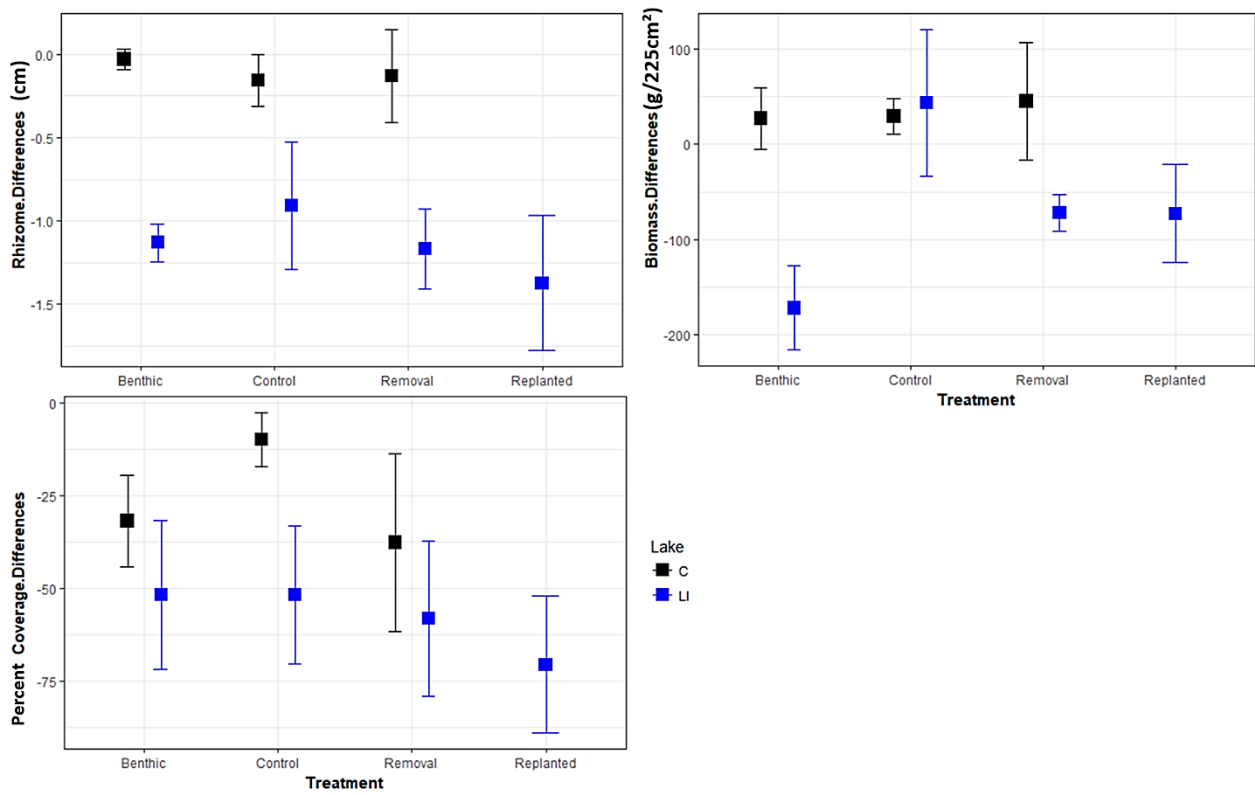
**Table 9:** Comparison of all 7 experimental plots in the Chestermere canal between 2016 and 2017 for the variables of rhizome size, biomass and percent coverage of plots by *B. umbellatus*.

	<b>2016</b>	<b>2017</b>	<b>ANOVA</b>
Mean rhizome size ±SE (cm)	3.2 ± 0.1	3.1 ± 0.1	$F_{2,10} = 0.9, p > 0.05$
Mean biomass ±SE ( $\log_{10}$ (g/225cm <sup>2</sup> ))	42.3 ± 4.5	75.5 ± 18.5	$F_{2,10} = 1.4, p > 0.05$
Mean percent coverage ±SE	35.2 ± 7.7	11.3 ± 2.9	$F_{2,10} = 9.5, p < 0.05$



**Figure 3.8:** For Chestermere Lake, (a) average rhizome size over 5 measurements  $\pm$  SE of each combined treatment before (2016) and after (2017) treatment, (b) plant dry mass (g/225cm<sup>2</sup>) of combined treatment plots before and after treatments, (c) percent coverage by *B. umbellatus* in each plot prior to treatment in 2016 and post treatment in 2017.

A two-factor nested ANOVA on the differences between 2016 and 2017 treatment parameters of rhizome size, biomass and percent coverage was analyzed from both lakes. Lake site did not have a significant interaction on treatments ( $F_{3,15} = 0.28, p > 0.05$ ) and was therefore no longer considered a variable. Rhizome size ( $F_{3,12} = 2.42, p > 0.05$ ), biomass ( $F_{3,12} = 2.09, p > 0.05$ ) and percent coverage ( $F_{3,12} = 1.22, p > 0.05$ ) at each lake indicated that all treatments had an insignificant effect in reducing *B. umbellatus* between years (Fig. 3.9a-c). Despite the statistically insignificant results, all measured variables did display decreasing trends between seasons. At Lake Isle, pairwise comparisons did show a greater difference between seasons but amongst the treatments, no one treatment proved to be more effective than the others. The difference in rhizome size (cm) was greatest at Lake Isle in the replanted plots ( $p = 0.12$ ) (Fig. 3.9a). Biomass (g/225cm<sup>2</sup>) difference was greatest with the benthic barriers at Lake Isle ( $p = 0.05$ ) (Fig. 3.9b). The replanted plots showed the greatest difference in the amount of regrowth (percent coverage) of *B. umbellatus* ( $p = 0.12$ ) (Fig. 3.9c).



**Figure 3.9:** Effect of treatments at both lake sites. (a) Average difference  $\pm$  SE in rhizome size (cm) (b) Average difference  $\pm$  SE in plant biomass (g/225cm<sup>2</sup>) (c) Average difference  $\pm$  SE in percent coverage from regrowth. “C” denotes Chestermere Lake and “LI” denotes Lake Isle. Benthic barrier mean effects are based on three plots at LI and two plots at C. Control means are based on three plots each from LI and C. Removal means are based on two plots from LI and C and the replanted mean is based on two plots from LI only.

### 3.4 Discussion

When *B. umbellatus* is abundant, it does not appear to be affected by crowding (shown by increasing percent coverage) or increasing depths of water. *B. umbellatus* experiences such rapid growth that populations rebounded between treatment years, resulting in insignificant declines in measured parameters in my experimental plots. Though not statistically significant, most treatment plots did show a decreasing trend in measured effects. There was a large effect of lake on the effectiveness of treatments with Lake Isle experiencing greater effects. The heavy growth of aquatic weeds covering the plots may have been acting in a similar manner to a benthic barrier, slowing the development of *B. umbellatus*. As overall trends saw a decrease in measured parameters, an increase in sample size and implementation of treatments earlier in the season may be more revealing. The idiosyncratic nature of the effectiveness of treatments is problematic for making overall recommendations to landowners and policy makers.

Despite the substantial removal of *B. umbellatus*, immediately following treatments there was substantial regrowth. This regrowth was either from missed plant material during removal or from plants dispersing from outside of the plots into the cleared area. Due to the sheer mass of the rhizome mat, it is more likely the regrowth was the result of rhizome fragments left in the substrate. The smaller population of *B. umbellatus* in the Chestermere canal made removal relatively more manageable and produced more significant results the following season compared to Lake Isle.

There are many reasons why treatments may have been insufficient to reduce *B. umbellatus* beyond a single season. The benthic barriers were left in place for 13-14 weeks which may not have been enough time to reduce rhizome reserves to the point where regeneration was not possible. As plants at the time of barrier placement were already mature, it is possible that barriers

may have been more effective if placed in the early spring when plants were small, immature and actively growing. The benefits of revegetation with indigenous plants were not observed to be effective due to low survival rates of transplants but replanting may not have occurred early enough in the growing season to allow these plants to become established (Toop and Williams, 1991, Hole, 1996).

Considering that treatments resulted in very little long-term reduction of *B. umbellatus*, annual treatments may be necessary at heavily infested sites. Therefore, it will be very important to consider hours of labour involved in each of the control methods. Removal of eight 4m<sup>2</sup> plots at Lake Isle took 16 hours with five people, while installing the benthic barriers required approximately five hours, which included initial cutting back and removal of leaf material from four plots. Benthic barriers provide the most time and cost-efficient method of controlling growth and may represent the control method with the most rapid effects. Benthic barriers have been used successfully in controlling Eurasian Watermilfoil (Laitala et al. 2012, Shaw et al 2016).

As no one control method appeared superior to the other, an integrated approach may be beneficial. Shaw et al. (2016) found that effective control strategies involved: a large initial investment into a control program; a program for early detection and rapid response to invasion; close ecological monitoring and commitment to a long-term control strategy. For *B. umbellatus*, an integrated approach could include cutting plants below the water line in deeper water and benthic barriers along shorelines. As benthic barriers seemed to affect plant growth, coverage of large areas with barriers, ideally as soon as the lake is free of ice and before the plants have broken dormancy, could be done and left in place for several seasons. Long term efforts with the involvement of many participants could also be effective, as demonstrated by the residents of

Camp Koinonia (Appendix D). Their yearly campaign to cutback *B. umbellatus* from boat docks and beach areas has reduced the rhizomes of the plants to the point that plants in the beach area are occasionally lacking rhizomes entirely.

Past campaigns by citizens to combat invasive species have proven successful, such as the Scotch Broom removal program in British Columbia. A website was established detailing the problem, how the community can be involved, and good news stories of Scotch Broom almost eliminated by some communities ("BroomBusters - Cut Broom in Bloom, Mid-Vancouver Island" 2017). By involving the public in research and education initiatives, a greater awareness of the societal impacts of science is created which may lead to a faster response in the form of partnerships and funding for needed projects (Dickinson, et al. 2012). This includes initiatives such as the creation of the cell phone application EDDMapS Alberta which is free to download (see <https://www.eddmaps.org/alberta/>). This application allows Alberta citizens to record and report invasive plant species in the province for real time tracking, province wide distribution and early detection of invasive plant species ("EDDMapS Alberta" 2017). These initiatives will be explored further in the next chapter and Appendix D.

## Chapter Four:

### Concluding Remarks on *Butomus umbellatus* in Alberta

Invasive nonindigenous aquatic plants are a major threat to aquatic biodiversity, causing the displacement of indigenous species (Ashton and Mitchell, 1989). *B. umbellatus* forms dense stands of vegetation that block sun light and decrease indigenous plant growth (which in turn decreases local animal species that rely on indigenous plants for food, spawning, or nesting material). The ecosystem services provided by the water bodies, such as recreational opportunities, are then reduced. Dense stands slow water flows and alter water oxygen levels while also changing the transport and deposition of sediments, especially in deeper waters, allowing the colonization of normally unsuitable areas by other plant species (Gunderson et al. 2016).

Knowledge of phenology of *B. umbellatus* as well as information on the costs of control measures are needed to find the life stage when invasive nonindigenous plants like *B. umbellatus* are at their most vulnerable to control methods (Buhle et al. 2005). Raising public awareness can decrease the spread caused by improper handling of plant material and by the clearing of indigenous plant stands. The goal of this study was to increase the knowledge of the natural history of *B. umbellatus* in Alberta, especially with regard to the interactive roles of ploidy and rhizome size in its invasion. I found that vegetative reproduction through rhizome fragmentation is a primary mode of reproduction in Alberta, and the extent of clonal reproduction may depend on ploidy.

Based on chromosome counts, correlating stomata guard cell lengths and pollen size and shape, most populations of *B. umbellatus* in Alberta appear to be the diploid cytotype, except for

the Innisfail area which contains the triploid cytotype. Despite being diploid (and presumably fertile), sexual reproduction (through seeds) was not the primary means of spread. In Lake Isle and the Lake Chestermere inflow canal, there was no evidence of *B. umbellatus* seeds in the seed bank, which would indicate control measures should focus on vegetative reproduction. These findings are consistent with the recent failed attempts to control *B. umbellatus* through removing inflorescences.

As plants have large carbohydrate reserves in the rhizomes, various control measures such as benthic barriers and cutbacks appear to cause the plants to draw on those reserves as indicated by a decrease in rhizome size and biomass in treated plants. However, the reserves in rhizomes were still able to regenerate the following year.

Manual removal of plants, though straightforward in execution, was surprisingly ineffective, likely because it was extremely difficult to ensure the complete removal of all plant material. Complete removal by methods such as suction harvesting, which was tested at the Chestermere canal, is promising but experiences the same problem (Appendix D). This would indicate that multi-season treatments, multiple treatments within a season, or the use of herbicides (Appendix D), are needed to ensure eradication of this species.

There are many parameters to consider if restoration of an ecosystem via its seed bank is to be relied on and research is lacking in this area once an invasion has been removed (Goodson et al. 2001). Depending on the composition of the seed bank, removing one invasive species may allow for a secondary invasion via the seed bank of another nonindigenous species (Gioria, et al. 2012). The canal bottom in Chestermere is dredged every winter which has prevented *B. umbellatus* from expanding into the canal, but dredging has failed to reach plants along the sides

of the canal. To avoid the potential problems of these wide scale removals, this study found that any control methods should begin in the spring when plants are still small, manageable and perhaps the most vulnerable to methods such as benthic barriers or herbicide applications. This would also increase the possible benefits of indigenous revegetation as new transplants would have the entire season to establish themselves and increase survivability over the winter. Ideally, early detection and removal of new populations of *B. umbellatus* is more desirable. This is shown to be more effective in controlling invasive species than attempting to control or reduce larger, established populations (Buhle, et al. 2004).

Control of *B. umbellatus* in Alberta will need a consistent, timely and seasonal approach that does not allow plants the time to replenish rhizome reserves or invest heavily into clonal reproduction. Engaging the public will be helpful in early detection and action, and informative brochures may raise awareness (see template for potential use by the Alberta Invasive Species Council; Appendix E). Management efforts will require a concerted effort between government, industry and the public, but increased collaborative participation will likely help combat this invasive species.

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## Appendix A:

### List of identified species in the research area of Lake Isle collected by Lisa Cahoon and identified by the U of C herbarium

Scientific Name	Common Name	Authority	Indigenous
<i>Achillea millefolium</i> , subsp. <i>lanulosa</i>	common yarrow	L., (Nutt.) Piper	Y
<i>Amelanchier alnifolia</i>	Saskatoon bush	(Nutt.) Nutt.	Y
<i>Canadanthus modestus</i> , syn. <i>Aster modestus</i>	great northern aster	(Lindl.) G.L. Nesom.	Y
<i>Carex aquatilis</i>	water sedge	Wahlenb.	Y
<i>Carex atherodes</i>	wheat sedge	Spreng.	Y
<i>Ceratophyllum demersum</i>	common hornwort	L.	Y
<i>Cirsium arvense</i>	Canada thistle	(L.) Scop.	N
<i>Cornus stolonifera</i>	red osier dogwood	Michx.	Y
<i>Epilobium ciliatum</i> , subsp. <i>ciliatum</i>	northern willow herb	Raf.	Y
<i>Equisetum arvense</i>	field horsetail	L.	Y
<i>Galeopsis tetrahit</i>	common hempnettle	L.	N
<i>Galium boreale</i>	northern bedstraw	L.	Y
<i>Impatiens capensis</i>	spotted jewelweed	Meerb.	Y
<i>Lemna trisulca</i>	star duckweed	L.	Y
<i>Lemna turionifera</i> , syn. <i>Lemna minor</i>	turion duckweed	Landolt, L.	Y
<i>Mentha arvensis</i>	wild mint	L.	Y
<i>Myriophyllum sibiricum</i>	Northern water milfoil	Kom.	Y
<i>Persicaria lapathifolia</i>	curltop lady's-thumb	(L.) Delarbre	Y
<i>Phalaris arundinacea</i>	reed canarygrass	L.	Y
<i>Pinus contorta</i>	lodgepole pine	Dougl. ex Loud	Y
<i>Populus tremuloides</i>	quaking aspen	Michx.	Y
<i>Potamogeton richardsonii</i>	Richardson's pondweed	(Benn.) Rydb.	Y
<i>Prunus pensylvanica</i>	pin cherry	L.	Y
<i>Prunus virginiana</i>	chokecherry	L.	Y
<i>Rosa acicularis</i>	prickly rose	Lindl.	Y
<i>Rubus idaeus</i> , subsp. <i>melanolasius</i>	North American red raspberry	L., Focke.	Y
<i>Rumex triangulivalvis</i>	willow dock	(Danser) Rech.	Y
<i>Sagittaria cuneata</i>	northern arrowhead	Sheld.	Y

<i>Salix discolor</i>	pussy willow	Muhl.	Y
<i>Schoenoplectus tabernaemontani</i> , syn. <i>Scirpus validus</i>	softstem bulrush	(C.C. Gmel.) Pallas, Vahl	Y
<i>Sonchus arvensis</i> , subsp. <i>arvensis</i>	field sowthistle	L.	N
<i>Sparganium eurycarpum</i>	broadfruit bur-reed	Engelm.	Y
<i>Spirodela polyrhiza</i>	great duckweed	(L.) Schleid.	Y
<i>Stuckenia pectinata</i>	sago pondweed	(L.) Borner. (Willd.) G.L.	Y
<i>Symphotrichum lanceolatum</i> , var. <i>hesperium</i>	western paniced aster	Nesom, (A.Gray) G.L. Nesom	Y
<i>Tanacetum vulgare</i>	common tansy	L.	N
<i>Typha latifolia</i>	broadleaf cattail	L.	Y
<i>Urtica dioica</i> , subsp. <i>gracillis</i>	stinging nettle	L., (Ait.) Selander	Y
<i>Veronica anagallis-aquatica</i>	water speedwell	L.	N
<i>Cladophora</i> sp. ( <i>Chlorophyta</i> )	filamentous green algae (mermaid's hair)	L.	Y
Cyanobacteria	blue-green algae		N

## Appendix B:

**List of identified species in the research area of the Chestermere canal collected by Lisa Cahoon and identified by the U of C herbarium.**

<b>Scientific Name</b>	<b>Common Name</b>	<b>Authority</b>	<b>Indigenous</b>
<i>Callitriche hermaphroditica</i>	northern water-starwort	L.	Y
<i>Chara sp.</i>	stonewort	L.	Y
<i>Potamogeton richardsonii</i>	Richardson's pondweed	(Benn.) Rydb.	Y
<i>Stuckenia vaginata</i>	big-sheathed pond weed	(Turcz.) Holub.	Y

## **Appendix C:**

### **Weed History at Camp Koinonia**

Our experience began in the early 60's at the start of the Camp. Our memories were preserved by a forward-thinking grandmother that bought one of the first movie cameras available.

I have a movie of the family swimming in clean water in front of our cabin circle in what looks to be 1962. There are also movies of boating at that time in an old wood speed boat and again the water is clean.

In the 70's my uncle built a weed cutter. I would call it a small crude model comparable to what we use at the lake today with a side mower attached to the front. I remember work bees when the weeds were cut in exactly the area in front of the swim area and piers I previously spoke of swimming in the 60's. This was only in the deep water (not shore). I water skied a lot in the 70's and always skied right into shore at the end of each run. There were no weeds that broke the surface of the water.

The weeds in the 80's was also not a problem on our shores. Fishing was great in the 60's, 70's and 80's.

The 90's and early 2000 saw an environmental shift in the thinking of the Camp with respect to weeds.

We were told weed cutting was not allowed and for the most part stopped.

We saw exponential weed growth in the 90's and early 2000's. We assumed the weeds were native. We obtained permits to keep our pier areas open and the two swim areas. The first at the end of loop #3 and the large area at the end of our point (boat launch area). This was also a time that the algae in the lake was noted to be increasing (the lake got greener earlier every year). The fishing also began to decline.

I can only estimate that we first had the flowering rush about 5 years prior to first discovering what it was. I say this because when it arrived the environmentalists told us it was native and could not be removed. We watched it for years as it grew and thickened into a mat. We watched the decayed plant matter being pushed into our shores by the ice for years. I would place an educated guess of 2007 being a time that it had already become enough of a mess that we were concerned.

With the flowering rush came a loss of all natural vegetation. The water was stale, the algae growth worsened. The water turned green earlier and the blue/green algae was found earlier and earlier every year to this date.

The cutting of this weed is currently the only reason our Camp Lake life has been sustained. Without cutting I fear our Camp would cease to exist as we know it.

Control and eradication are key to the survival of this and many other lakes.

Doug Buchholtz  
February 24, 2017

## Appendix D:

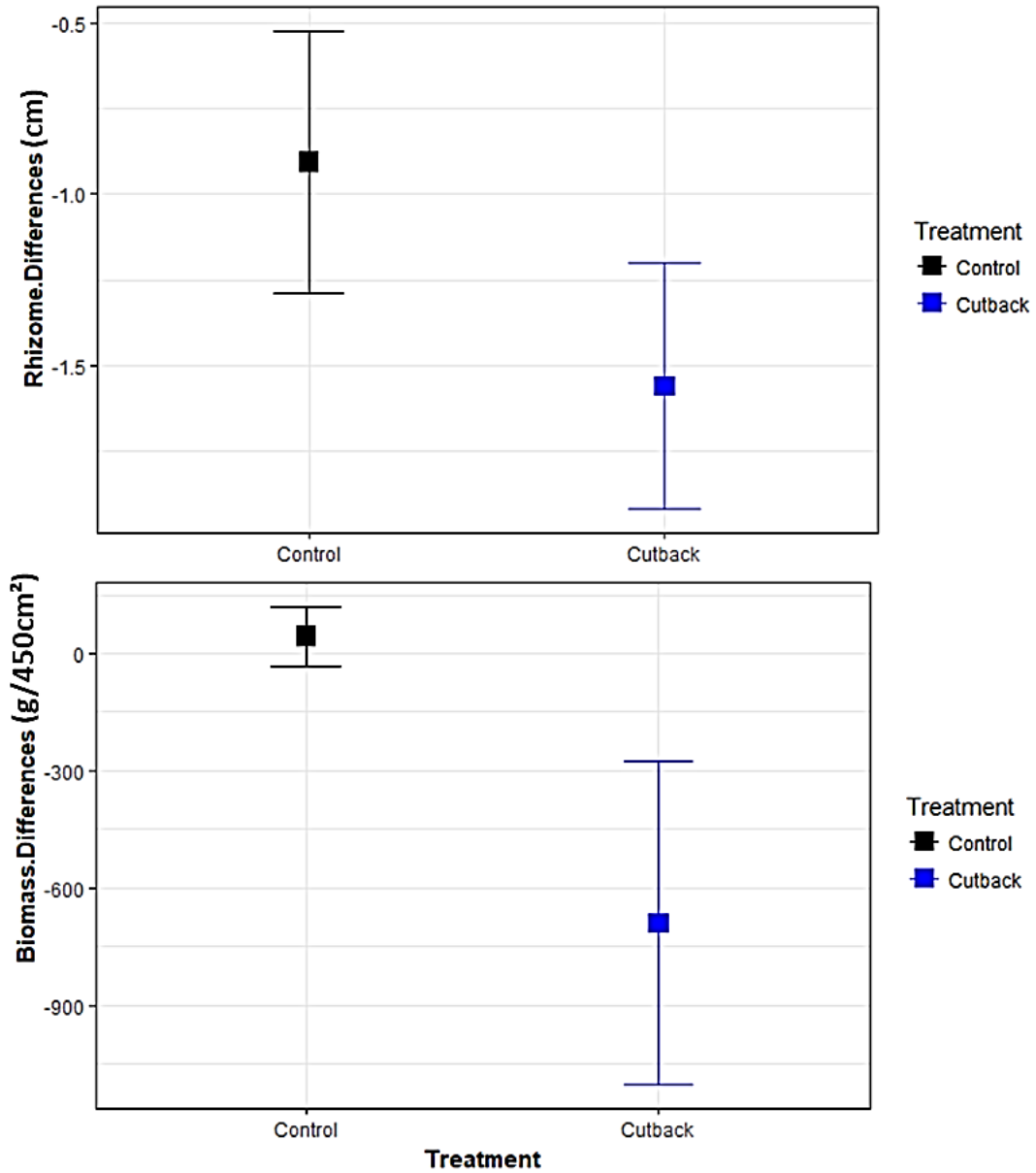
### Other control methods of *B. umbellatus* at Lake Isle

#### D.1 Plant cutbacks

One of the most important outcomes of this study was the air of collaboration that was generated through many hours of informal consultations with landowners. When the experimental plots were being established at Lake Isle in 2016, conversations with lake residents piqued their interest in determining whether their ongoing efforts to reduce Flowering Rush were having a noticeable effect. While not a formal part of my research proposal, I embarked on an *ad hoc* observational analysis of whether regular cutting of above-substrate parts of the plant reduced rhizome biomass.

I took 10 rhizome measurements and four plant biomass measurements from the boat launch and beach areas where residents have been cutting Flowering Rush below the waterline each year for the last 20 years (the “cutback” site). By comparing the rhizomes to my measurements from other sites, I could examine if their efforts would reduce photosynthetic rates enough that plants would draw upon, and thus reduce, rhizome carbohydrate reserves. Further west of the beach area by 0.4km was an area where no alterations of *B. umbellatus* had taken place (a “control” site). A mechanical harvester was used on the western site on two separate occasions after pre-sampling of plants occurred. I was able to collect, six pre-treatments (2016) and six post-treatment (2017) rhizome samples and four plant biomass measurements from each year to examine the effects of mechanical and manual harvesting compared to control plants. I compared the average rhizome size of cutback areas to the average of non-cutback rhizome measurements  $\pm$  standard error (Fig

D.1). I also compared the average biomass of cutback plants to the average biomass of non-cutback plants (Fig. D.1)



**Figure D.1:** Manual and mechanical harvesting cutback effects on (top) rhizome size (cm  $\pm$  SE) and (bottom) biomass (g/450 cm<sup>2</sup>  $\pm$  SE) vs. non-cutback (control) plants.

A two-sample t-test was performed on normally distributed pre-treatment data from plants collected from non-cutback areas (experimental plots and pre mechanical harvest areas) and the cutback areas of the beach and boat launch in the summer of 2016. A significant difference was seen in rhizome size between cutbacks and non cutbacks (Mean  $\pm$  SE: 2.0cm  $\pm$  0.7cm and 4.6cm  $\pm$  0.1cm respectively,  $t_{17} = 4.40$ ,  $p < 0.0001$ ). These results indicate the plants do draw on rhizome reserves after repeated cutbacks. It was noted that the plants in the beach area specifically, were sparse and had little to no rhizomes and were anchored in the substrate by roots only. For the area cut by the mechanical harvester, a paired t.test was used to determine any difference in rhizome size after cutting. In just one season of cutting, the rhizomes did shrink in size with a mean difference of 1.14cm between seasons ( $t_4 = 5.66$ ,  $p < 0.05$ ). Biomass decreased by 34%.

Residents felt the mechanical harvester was to blame for the rapid spread of Flowering Rush throughout the lake. As the harvester floats and the blades only cut the leaves, it is unlikely the harvester was responsible for the spread of Flowering Rush. The mechanical harvesting of plants may be beneficial but this would need to involve regular cut downs of the plants to insure the leaves remain below the water line, hampering the plants' ability to photosynthesize.

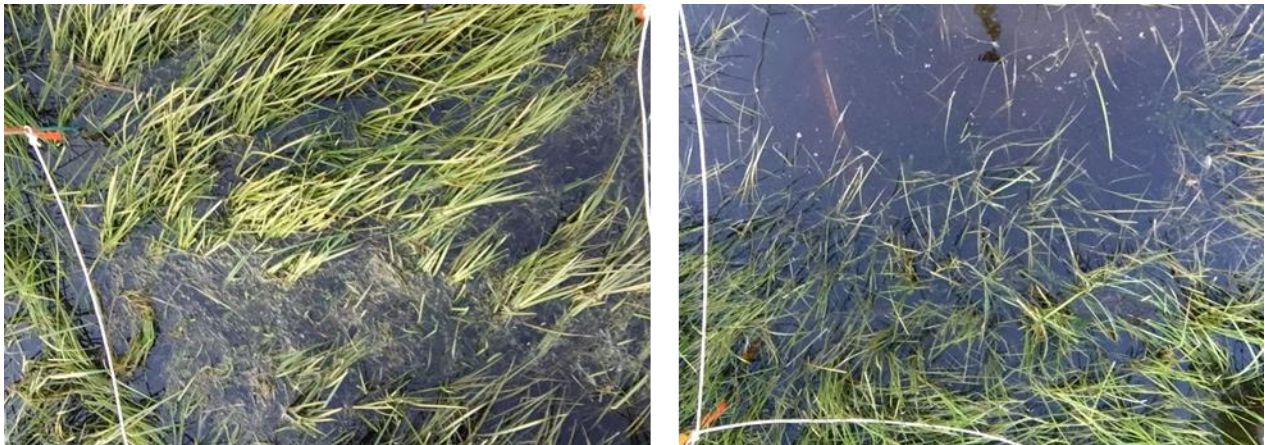
## **D.2 Herbicide trials**

Other drastic measures for removal of *B. umbellatus* are currently being considered. Widespread herbicide use is a potential control method that shows some promise. Besides the negative public perceptions surrounding herbicide use, employment of this method will need to take care to ensure the mass die off of plants, and the consequent reduction in oxygen from the water, does not result in fish kills (Jewell, 1971, SePRO, 2017).

September 2016, plots were set up by Alberta Environment and Parks for the testing of two different herbicides. Four 2m x 2m plots, alternated with five 2m x 2m control plots, were established approximately two meters from the shoreline (site A). A second similar configuration was established approximately 100m further west of site A (site B). Site A was sprayed with the aquatic herbicide imazapyr (Habitat Aqua™) which was brought into Canada from the USA under an emergency use application. Imazapyr is a systemic herbicide not currently licensed in Canada. It functions by inhibiting the synthesis of the enzyme, acetolactate synthase, preventing growth (WDNP, 2012). Plant death and decomposition occurs gradually over several weeks to months. Site B was sprayed with Canada's only licensed aquatic herbicide, diquat (Reward™). Diquat is a contact herbicide which disrupts cell membranes, interfering with photosynthesis. Plants sprayed with diquat appear burned within three days of application (WDNP, 2012). The herbicide plots would be re-evaluated the following season for regrowth of *B. umbellatus*.

While effective, herbicides can damage the environment and therefore require permits that can delay or prevent their regular use. Due to required licensing, permissions and community notifications, spraying did not occur until after the flowering season (September 19<sup>th</sup>, 2016) and this compromised pre-treatment plant collection and evaluation. Two weeks after the herbicide applications, 100% of the diquat treated plants were burnt below the water line. Imazapyr treated plants were still present but 85% appeared to have blackened tips. Because no information was collected on percent coverage of *B. umbellatus* in each plot, observations the following year concentrated on growth characteristics of individual plants between diquat and imazapyr plots. Notably, all treated plots had abundant *B. umbellatus* the following year (Fig D.2). Plants in the diquat plots were slower to emerge than the imazapyr treated plots and appeared to have a burned

appearance to the tips. Visually, the regrowth of *B. umbellatus* was diminished from surrounding untreated plants by 10-30%. Plant regrowth in imazapyr treated plots appeared undiminished from surrounding untreated plants (Fig D.2) however, imazapyr treated plants did appear more yellow in colour than untreated plants, indicating a possible disruption to the production of chlorophyll needed for photosynthesis. Further studies should be performed to determine the efficacy of either herbicide. However, as imazapyr is not currently licensed for use in Canada, efforts would be better spent on studying the effects of diquat. Several years of herbicide testing has been undertaken in Minnesota using diquat with promising results (Madsen et al. 2014).



**Figure D.2:** Comparison of regrowth of plants treated with imazapyr (left) and plants treated with diquat (right) the previous season. Observationally, diquat plants were slower to emerge and appeared to be less dense than imazapyr treated plants by 10-30% when compared to the growth of untreated plants in the area. Imazapyr treated plants were yellow in colour compared to diquat plants and the surrounding untreated plants.

### D.3 Suction harvesting trial

In the inflow canal at Chestermere Lake, a trial area was cleared of rush via suction harvesting the summer of 2016. Observations on regrowth were made the following summer. Suction harvesting involves the removal of substrate from around the plants allowing for removal of the entire plant either by hand or by suction as well. The suction harvest area was observed to have some regrowth of Flowering Rush, especially further out into the canal, but did appear to be reduced from the year before (Fig D.3).



**Figure D.3:** The picture on the left shows the consistent density of Flowering Rush growing along the Chestermere canal in 2016. The picture on the right is the area which underwent suction harvesting in the summer of 2016, showing the regrowth in 2017 which appears to be reduced.

## Appendix E:

Flowering Rush brochure created for potential use by the Alberta Invasive Species Council



**Current control efforts in Alberta**

**What is being done?**

Research is ongoing in Alberta to determine the growth habits of Flowering Rush and the best ways to control/eradicate and prevent further spread.

Measures include:

- Benthic barriers
- Complete removal of plants with and without native plant revegetation
- Cutbacks below the water line
- Suction harvesting

Ongoing herbicide trials are also in place to determine the effectiveness of this method of control.

**Alberta Invasive Species Hotline:**  
1-885-336-2628

**Contact Us:**  
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17507 Fort Road NW  
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Email: [info@abinvasives.ca](mailto:info@abinvasives.ca)  
Web: [www.abinvasives.ca](http://www.abinvasives.ca)



*By working together, we can help stop the spread of Flowering Rush.*

**What can you do to help stop the spread of Flowering Rush?**

- Report any suspected sightings.
- Ensure boats are clean, drained, and dry to prevent the spread of bulbils and rhizomes.
- Pluck any floating sprouted plants out of the water and place in the garbage.
- If digging out small populations on water front property, ensure all rhizome material is removed by feeling through the soil with your hands. Make sure there are no fragmented floating rhizomes or bulbils after digging is complete.
- Avoid walking on or among Flowering Rush colonies as this can fragment rhizomes and detach bulbils.
- Avoid the use of chemical fertilizers on lakeside properties which drain into lakes providing extra nutrients for Flowering Rush and other weeds.
- Flowering Rush is easiest to deal with in Spring when the plants are small and more manageable.

**FLOWERING RUSH (BUTOMUS UMBELLATUS) IN ALBERTA**

*A serious threat to our lakes and waterways, but you can help*



The flowers with a backdrop of flowering rush leaves.



Newly sprouted plants



Fleshy rhizome with proformed roots



Stem cross section.

## Flowering rush in Alberta

The aquatic perennial plant *Butomus umbellatus* L. or Flowering Rush, is a native of temperate Eurasia. Since arriving in Canada as a horticultural plant, it has spread rapidly across Canada and aggressively colonizes a variety of habitats. It can grow emergent on the shore line to completely submerged in water up to 7m (23') deep. In Spring, rhizomes begin to sprout much earlier than native vegetation.

Flowering occurs from early summer to mid-fall. Once mature, leaves collapse and begin to decompose taking oxygen from the water.

### How to identify flowering rush:

Resembling a large sedge, upright foliage can reach 2m (7') in height. When first emerging, the base of leaves are usually

*Flowering rush is a prohibited noxious weed in Alberta and threatens the very existence of our lakes and waterways.*

a dark red in colour. Leaves are triangular, spongy and compressible and spiral near the top. Roots are fleshy and rhizomatous and can produce many bulbils. Flowers, growing in an umbel, are light pink in colour.

### Why is Flowering Rush a problem for our lakes and waterways?

- Displaces native vegetation in an area.
- Colonizes previously non vegetated areas.
- Plants spread rapidly via rhizome fragments and bulbils.
- Slows water flows, lowers water oxygen levels which can lead to fish kills.
- Diminishes nesting areas for water fowl and spawning areas for some fish species.
- Clogs boat propellers and interferes with recreational water use.



Bulbils which develop into complete clones of the parent plant. They are buoyant and can travel long distances.

- Alters transport and deposition of sediments.
- Changes shorelines of water bodies.
- Irrigation canals can be choked out by flowering rush, reducing water flows, clogging trash racks, intake pumps and impacting the businesses they service.
- Dense flowering rush stands harbour the great pond snail, which hosts the parasite responsible for swimmer's itch.



Flowering rush infestation in an Alberta Lake.