THE BIOLOGY AND MANAGEMENT OF *STRATIOTES ALOIDES* IN THE TRENT RIVER, ONTARIO

A Thesis Submitted to the Committee on Graduate Studies
in Partial Fulfillment of the Requirements for the Degree of Master of Science
in the Faculty of Arts and Science

TRENT UNIVERSITY

Peterborough, Ontario, Canada

Environmental and Life Sciences M.Sc. Graduate Program

May 2017

ABSTRACT

THE BIOLOGY AND MANAGEMENT OF *S. ALOIDES* IN THE TRENT RIVER, ONTARIO

by Robert Canning

Invasive aquatic plants can create negative ecological, economic and social impacts when they displace local vegetation, interfere with shipping and navigation and inhibit water-based recreational activities. In 2008, the first North American occurrence of the invasive plant *Stratiotes aloides* (Water soldier) was identified in the Trent River, Ontario. This research measured offset photosynthesis and turion germination to determine the light compensation point (5.2-5.4m) and maximum depth of colonization (4-6m) for *S. aloides* propagules using *in situ* incubations and controlled growth experiments. The effects of spring and fall chemical (Diquat) and physical (hand raking) treatments on *S. aloides* biomass, local macrophyte recovery and community dynamics in the Trent River were also measured. The target of a 75% minimum reduction in *S. aloides* biomass was not attained using any of the treatment methods and no perceivable recovery of the local plant community was observed. Significant *S. aloides* regrowth was recorded for both treatment methods regardless of application timing.

Key Words: Stratiotes aloides, Water soldier, aquatic macrophyte, chemical control, manual control, invasive aquatic species, photosynthesis, maximum depth of colonization

ACKNOWLEDGEMENTS

I would like to thank my supervisor Dr. Eric Sager for the guidance and encouragement needed to complete this project and my thesis committee Dr. Joanna Freeland and Dr. Tom Whillans for their support and patience throughout this process. I would also like to thank Trent University, the Ontario Ministry of Natural Resources and Forestry and the Invasive Species Centre for their funding and technical support of this project. I would like to thank my loving and understanding family for giving me the kick in the pants I needed to get me on track again. Thanks to Caroline Wylie and Nick Weissflog for helping with field sample collection and biomass processing. Lastly, I would like to thank my good friends Kyle Borrowman for initiating me into the world of aquatic plants and Colin Cassin for never asking about it.

Table of Contents

List of Figures

List of Tables

CHAPTER 1: *S. aloides* in Ontario

1.1 Introduction

Aquatic macrophytes, plants that grow entirely or partially underwater, serve as one of the fundamental cornerstones to a diverse and functioning freshwater ecosystem. They not only improve the productivity and change the physical and chemical properties of the system they are present in, but they form complex relationships with and are essential to the survival of a great number of organisms within their ecosystems (Anton-Hough et al, 1989; Caraco et al, 2006; Carpenter and Lodge, 1986). These plants can interact with their environment in a variety of ways. At the organism level, aquatic plants can serve as source of shelter, food and a medium for the reproduction of fish and macroinvertebrate species. They can also influence physical characteristics within the ecosystem by altering water temperature, clarity and flow. Additionally, macrophytes can influence biogeochemical processes such as nutrient cycling and oxygen availability dynamics (Barko et al, 1991; Carpenter and Lodge, 1986). Healthy aquatic plant communities directly benefit humans in a number of ways including: providing a traditional food source, improving the quality of hunting and fishing resources and filtering water borne contaminants (Millenium Ecosytem Assessment, 2005; Newman, 1991; Wang et al, 2014).

The growth of macrophyte communities can be limited by a number of variables, but most commonly nutrient availability, light exposure and interspecies plant competition for resources (Chambers and Kalff, 1985; Van den Berg et al, 2003). In an ecosystem where there are multiple co-existing plant species, the growth of these macrophytes is kept in balance through their struggle for access to limited resources and as a result, a relatively stable system is maintained (Van den Berg et al, 2003). Just as a lack of aquatic vegetation can inhibit the establishment of a complex ecosystem, excessive plant growth can decrease the overall health and diversity of that same system.

A single species that disproportionately dominates its environment and spreads uncontrollably beyond its traditional geographic range can be considered invasive. Such species can affect ecosystems in unpredictable and negative manners through the displacement of other organisms, creation of monospecific plant communities, disruption of waterway navigation and losses in recreational and commercial opportunities (Havel et al, 2015). Invasive plants can be both indigenous to that location or exotic species (introduced). The focus of this thesis is on introduced, invasive species.

In the United States there are ~17,500 species of native terrestrial and aquatic plants and over 5000 species of non-native plants, around 40 of which are considered invasive aquatic plants (Bergeron et al, 2008). Between the costs of management activities, damages to ecosystems and other associated economic losses, the cost of invasive aquatic plants in the United States was estimated at $110 million and the total annual cost of all invasive species (aquatic and terrestrial) was $137 billion in 2000 (Pimentel et al, 2000). In Canada, 486 invasive plant species have been documented. The highest concentration is found in Ontario, where 91% of all of Canada’s invasive plants are located, including 19 species of aquatic plants (OMNRF, 2013). In 2006, estimated economic costs from aquatic invasive species in Canada ranged from $298 to 776 million/year, with total invasive species costs to Canada’s economy estimated at $34.5 billion per year (Colautti et al, 2006).

Exotic invasive aquatic plants can enter ecosystems through both intentional and accidental release and natural diserpsal. Some of the most common vectors for transmission are the agricultural and horticultural industries, international shipping and other forms of transportation (Havel et al, 2015). Once established in a native system, they can be extremely difficult, if not impossible, to eradicate. With the economic costs and ecological impacts of these species clearly documented (Lovell and Stone, 2005), preventing the introduction of these plants as well as immediately acting to manage the spread of new species are essential to preserving natural biodiversity and limiting the widespread negative effects of invasive aquatic plants.

To mitigate the impacts of invasive aquatic plants, ecosystem managers can use a variety of control and eradication techniques designed to reduce or eliminate a problem species. These may include chemical and physical measures including: herbicide application, mechanical cutting and releasing biological control agents (Haller, 2014; Netherland, 2014; Simberloff, 2003). Matching appropriate types of control actions to each individual species and the locations in which the management will take place can help to improve treatment efficacy (refer to section 3.1 for a more detailed overview of aquatic plant control options).

1.2 The Arrival of *Stratiotes aloides*

In 2008, a non-native, invasive aquatic plant named *Stratiotes aloides* (Water soldier) was discovered growing in a section of the Trent-Severn Waterway (TSW) in Ontario called the Trent River. This was the first documented occurrence of this plant being exposed to and persisting within a natural ecosystem in North America, but is native to and has populations across parts of Europe and Asia. Although not conclusive, the arrival of *S. aloides* was attributed by the local residents to the unintentional release of ornamental plants in a water garden adjacent to the Trent River (Canning, pers. comm.). After its appearance, the growth rate and competitive nature of this plant were quickly realized as it spread throughout sections of the waterway and began to dominate local plant communities (Canning, 2012). In response to the discovery of *S. aloides*, the Ontario Ministry of Natural Resources and Forestry (OMNRF) designated the plant as an invasive aquatic species that poses a threat to Ontario's environment, economy and society (OMNRF, 2013). In 2013, the Council of Great Lakes Governors and the Premiers of Ontario and Quebec identified 16 aquatic invasives that hold the highest priority as "least wanted" species, including *S. aloides*. Before its recent appearance in Ontario, the potential invasiveness of this species had already earned it a proactive designation as a federally controlled noxious weed in several US states and in Australia (Rasmussen, 2000; USDA, 2014; van Oosterhout et al, 2013).

The severity of the consequences of *S. aloides* escapement into Ontario is two-fold. Not only is this plant capable of drastically altering an ecosystem by converting native plant communities into a monoculture of *S. aloides*, but the fact that this plant has never previously been encountered in North America means that there is a critical lack of information regarding the biology of this species in this invaded region, its ability to establish in other water bodies of Ontario, and how to effectively manage, and ultimately, eradicate this species. In contrast to the situation in Ontario, this species has undergone widespread decline in much of its historical distribution in northern Europe, such that local management priorities now focus on its conservation, not eradication (Smolders et al, 2003; Suutari et al, 2009).

At the time this research was initiated (2013), *S. aloides* had been present in the TSW for four years and had been able to grow, for the most part, unchecked by control actions because no clear methods for treatment could be identified. The size of the entire population at that time was ~50 ha. The first-time nature of this introduction meant that no herbicides in Ontario were approved for application to these plants or in the Trent-Severn Waterway and there were no biological control options available. The size of the entire population at that time was ~50 ha. At present (2016), *S. aloides* has travelled over 20km downstream from its point of introduction and has colonized over 200 ha of water within the TSW (OFAH, 2016).

In dealing with invasive species, it has been recognized that once the opportunity for early eradication has passed, the importance of understanding the biology of the species increases as a species becomes ever more deeply rooted within its new ecosystem (Simberloff, 2003). Biological research directed towards key knowledge gaps such as (in the case of *S. aloides*) mechanisms of reproduction and spread and the role of competition and environmental conditions towards limiting population growth, can provide valuable insights to inform the long-term management/eradication of the species.

With a fast growth rate and excellent competitive abilities (Cook and Urmi-Konig, 1983; De Geus-Kruyt and Segal, 1973; Erixon, 1979) the potential risk to native ecosystems posed by *S. aloides* means that management action should follow its introduction and be informed by biological research. Examples of other invasive plant introductions such as *Crupina vulgaris* (common crupina) in the U.S in 1968, *Caulerpa* *taxifolia* (alga) in Monaco in 1984 and *Clidemia hirta* (Koster's curse) on Hawaii in 1941, where much biological research was carried out, but went unsupported by management action, should serve as cautionary tales (Simberloff, 2003). Early biological information that identified susceptibilities of these three species to available control techniques was ignored and treatment actions were delayed and cancelled in some cases. A common theme of these introductions was that the government and non-governmental organizations (NGO) dealing with the introduced species avoided taking responsibility for the problem and were unable to agree on a course of immediate action (Simberloff, 2003). In the face of indecision, they argued that more research was needed to assess how severe the species introduction would be and no attempts were made to try and treat the species with the basic biological information available at that time (Simberloff, 2003). In these cases, thousands of hectares of additional land were colonized by these species and the longevity of their invasions was extended before any treatment was administered (Mack and Lonsdale, 2002; Meinesz, 1999; Simberloff, 2003). Management actions can be informed by both the species biology and the best-suited treatment options given the environmental characteristics of the site, but the longer an invasive species is present within an ecosystem, the harder and more costly it becomes to remove it (Pimentel et al, 2000; Simberloff, 2003).

1.3 Literature Review

1.3.1 Biological Classification

Kingdom	Plantae
Division	Magnoliophyta
Class	Liliopsida

Order	Hydrocharitales
Family	Hydrocharitaceae
Genus	Stratiotes L.

(USDA, 2014)

S. aloides is in the same family (Hydrocharitaceae) as a number of well-known, highly invasive aquatic plants such as: *Egeria densa* (Brazilian Waterweed), *Hydrilla verticillata* (Hydrilla) and *Hydrocharis morsus-ranae* (European Frog's-Bit).

1.3.2 Morphology

S. aloides plants grow in a rosette formation (**Figure 1**) and have leaves that are long and thin and range in color from light to dark green. The mature, outer leaves of the plant can reach lengths in excess of 50 cm (Canning, 2012; Toma, 2006), yielding plants with diameters greater than 100 cm. Leaf shape varies depending on plant growth type with emergent plants having straight leaves and submerged plants typically exhibiting spirate leaves (**Figure 2**) (Toma, 2006). All plants have spines that grow on the edges of the leaf blades on both new and mature leaves, which become more prominent as the plant increases in size. The spines are 2-5.5 mm in length (Cook and Urmi-Konig, 1983) and are present on the leaf at a frequency of about 4 per every centimeter of leaf (Toma, 2006).

Figure 1:Early drawing (1891) of a *S. aloides* rosette showing serrated leaves, roots and flower structure (Masclef, 1891-reproduced with permission by Wikimedia Commons)

Figure 2: Emergent *S. aloides* rosette (Opioła, 2009- reproduced with permission under GNU Free Documentation License and Wikimedia Commons)

The roots of *S. aloides* plants are long, white and thin, averaging between 60-80 cm in length on full-grown plants (Toma, 2006). The ends of these structures are covered in fine root hairs, which are buried in the sediment (Smolders et al, 2003). The roots are used primarily as a support structure (as opposed to nutrient uptake), and only loosely hold the rosette in the sediment (Tor Nielson and Borum, 2008).

S. aloides plants produce three types of reproductive structures: seeds, clonal offsets and turions. The seeds are cylindrical in shape with a slight inwards curvature (Cook and Urmi-Konig, 1983). They are around eight mm in length and two mm in width. Seed production is highly variable between *S. aloides* plants in a population and seed output has been observed to range between nine and 1863 seeds per 100 plants, with the average of 10 seeds per plant (Smolders et al, 1995b). In Ontario, seed production has not been observed and it is believed that these populations are fully reliant on vegetative reproduction.

Offsets (**Figure 4**) are clonally produced from their parent plants and use connecting stolons (ranging from eight to 15 cm in length) to support their early stages of development. Once sufficiently advanced, the stolon decays and releases the offset (Renman, 1989a). Mature plants can typically support between one and six clonal offsets at any given time during the growing season, but plants with over 20 offsets have been recorded (Toma, 2006, 2012).

Turions are overwintering, axillary buds that are produced around the basal node of the plant and are released as the rosette decays in the fall and winter. They are teardrop shaped and can be either flat or round sided (**Figure 3**). The average length of mature turions is around 2.25 cm and the average fresh weight around 0.46 g (Canning, 2012). *S.*

aloides plants, on average, can support between one and three turions at any given time during their production period (Toma, 2006, 2012), but the presence of eight at one time has been observed (Canning, 2012).

Figure 3: Juvenile *S. aloides* offset (right) and grouping of mature turions (left) collected from the Trent River. Note the formation of the stolon at the base of the offset, while turions are attached directly to the base of the mother rosette

Figure 4: Progression of *S. aloides* offsets from immature "bud" to fully formed juvenile rosette. Specimens collected from the Trent River population

1.3.5 Life Cycle

S. aloides plants start the spring growing season resting on the bottom, where they develop roots and produce new leafy biomass. Since *S. aloides* retains a large percentage (~60%) of its peak summer biomass while overwintering, these plants can retain nutrients for extended periods of time compared to other species (Strzalek and Koperski, 2009). When enough resources have been allocated, the plants start to produce offsets, which they will continue doing throughout the growing season until the onset of winter. As the production of offsets and biomass continues, a large percentage of *S. aloides* plants will begin a seasonal transition and gradually rise up the water column until they form a floating/emergent mat of plants.

The seasonal movements of *S. aloides* plants are controlled by their specific gravity (Cook and Urmi-König, 1983). Specific gravity, in this case, can be defined by the density of *S. aloides* plants compared to that of an equal volume of water. In the spring, new growth traps gasses within the interstellar spaces of the leaves, which lessens the specific gravity compared to the water and allows the plants to float to the surface. In the late summer and towards the end of the growing season, when the majority of leaves left on *S. aloides* plants are older and senescent (Renman, 1989a), these interstitial spaces collapse and fill with water, causing the plants to sink (Cook and Urmi-König, 1983). Plants that are unable to produce sufficient new growth during the beginning of the growing season will remain close to the bottom for the season and have been found to behave somewhat differently than emergent plants in terms of offset production (Toma, 2006). Emergent plants have been found to remain attached to their offsets for a shorter period of time (~ three weeks) compared to the submergent plants, which were found to retain offsets on the stolons for up to three months. These offsets were at a more advanced stage of development, based upon leaf length, when they were released (Renman, 1989a; Toma 2006).

Conflicting reports have been published concerning characteristics related to *S. aloides* dimorphism/ecomorphism (its existence in submerged and emergent forms). Periodically, scholars have attempted to address the confusion and establish a new baseline of accurate information (Cook and Urmi-König, 1983; Efremov et al, 2015; Efremov and Sviridenko, 2008; Tor Nielson and Borum, 2008). Historically, the plant's submersed and emergent forms have been reported to exist separately (Erixon, 1979; Renman, 1989a). It was believed that this dimorphism occurred because turions become submerged plants while offsets become emergent plants (Kornatowski, 1983). Renman (1989a) reported that unlike turions produced by submerged plants, turions arising from emergent plants did not successfully overwinter in Sweden. Similarly, Erixon (1979) studied a colony of submersed *S. aloides* in Sweden and reported that their turions successfully overwintered (Erixon, 1979). Renman (1989a) concluded that the submerged form of *S. aloides* occurs in deeper water and retains nearly twice as much biomass as the emergent form during the winter. Weissflog and Sager (2016) observed significant density and biomass increases when sampling emergent versus submerged *S. aloides* populations. Toma (2006) studied emergent and submersed forms of *S. aloides* (growing in the same body of water in Poland) and concluded that there were significant differences in morphology and anatomy between forms. The submersed form has twisted light green leaves that are longer, more flexible, and thinner than the emergent form. Leaves of the emergent form are darker green, straight, and contain many stomata (Toma, 2006). The capacity of a single plant to exhibit both submersed and emergent forms at different times of year was documented by Cook and Urmi-König in 1983. Tor Nielsen and Borum (2008) suggest that the observations of exclusively submersed colonies of *S. aloides* by Erixon (1979) and Renman (1989a) occurred in clear waters with low levels of available nutrients. They conclude that most *S. aloides* populations shift from submersed form during the winter to emergent form in the spring through fall (Tor Nielsen and Borum, 2008). Toma's (2006) account of *S. aloides* dimorphism concludes that differences in nutrient availability played no role in differences in plant size observed.

By mid-summer, the focus for *S. aloides* is primarily biomass and offset production, but it is at this time that mature individuals will flower (**Figure 7**) and attempt to produce viable seed for a period of approximately four to five weeks (Renman, 1989a). Although this species is commonly cited as dioecious in the literature (De Geus-Kruyt and Segal, 1973; Tor Nielson and Borum, 2008), rare occurrences of selfpollinating plants have been documented (Cook and Urmi-König, 1983; Forbes, 2000; Richards, 1986). This happens when a female plant develops fertile stamens on the outer whorls of its flowers (Cook and Urmi-König, 1983). These plants are classified as subgynoecious, meaning that only some of the flowers on a plant can self-pollinate (Richards, 1986). In regions where both male and female plants are present, pollination occurs through insects (entomophily) (Tanaka et al, 2004) and the pollination distance is limited. Successful pollination of *S. aloides* plants requires that female and male individuals are no farther than 1000 m apart (Smolders et al, 1995a). Female flowers can still produce fruits with or without the presence of male plants, although these fruits are always parthenocarpic. Seed presence can be estimated through the measurement of fruit size, with seed bearing fruits ranging from 17-20 mm in length and seedless fruits being ten millimeters or less (Smolders et al, 1995b).

In the late summer/early fall, turion production commences and continues alongside offset production until mid-winter. Turions dispersed at this time germinate and appear in the following spring as miniature rosettes (Renman, 1989a). There seems to be some potential for the reproductive type in which a rosette began its life to influence the plants' mature growth form, as turions have been shown to develop into submerged plants and offsets to develop into emergent type plants when grown under the same environmental conditions (Kornatowski, 1983). As the growing season passes and temperatures fall, *S. aloides* plants complete their seasonal transition and return to the bottom of the water column as their decaying roots and leaves increase their specific gravity and pull the plant rosettes down to the sediment for the remainder of the winter.

Figure 5: *S. aloides* flower showing petal arrangement and reproductive organs. This specimen was collected on the Trent River and was brought into a climate controlled environmental chamber where it began to flower. In Ontario, flower production in the field is rare and seed production has not been documented

Although *S. aloides* can reproduce both sexually and vegetatively, sexual production has not been observed in Ontario (flowers were documented for the first time in 2014) and is increasingly rare within its native range due to population decline (Cook and Urmi-Konig, 1983; Smolders et al, 1995a, 1995b), leaving vegetative propagation as this species' most important form of population recruitment. Despite its reliance on offsets and turions, this species is capable of producing impressive quantities of biomass and the lack of sexual reproduction is generally not seen as an impediment to population expansion. Accounts of this species in Holland state that once introduced, *S. aloides* was able to almost completely colonize and establish 100% cover of an inland lake in less than three years (Erixon, 1979). Quantitative measurements of show the average productivity of this species at between 112-210 kg ha^{-1} day^{-1} (dry weight), with maximum productivity reaching up to 460 kg ha^{-1} day^{-1} (dry weight) (De Geus-Kruyt and Segal, 1973). This species has the ability to accumulate large quantities of biomass and the standing crop mass of *S. aloides* in bodies of water in the Netherlands was estimated at 6300 kg ha^{-1} dry weight (De Geus-Kruyt and Segal, 1973; Erixon, 1979). In Ontario, standing crop biomass has been estimated as high as 8710 kg ha^{-1} dry weight in high-density areas (Canning, 2012).

S. aloides is primarily reliant on hydrochoric dispersal to expand its distribution (Sarneel, 2013; Smolders et al, 1995a). The buoyancy of seeds and turions has been well documented and once released, these propagules can remain floating for many days. (Forbes, 2000; Renman, 1989a; Smolders et al, 1995a)**.** Turions in particular have been documented to remain buoyant for over 6 months following release (Sarneel, 2013) Mature plants, especially in their emergent form, dislodged by wind and current action, can drift throughout a water body into new, previous unreachable locations. In an open, flowing system like the Trent River, hydrochoric dispersal of propagules is likely the primary means by which this plant can transmit propagules over long distances. Effective water borne transport could see viable *S. aloides* turions and offsets carried beyond the Trent-Severn Waterway and into the Great Lakes.

Although there is potential for endozoochoric dispersal of propagules (Forbes, 2000; Smolders et al, 1995b**)**, no conclusive evidence for this exists. Seeds are more likely than turions to be dispersed by animal vectors because the inside of *S. aloides* fruits contain mucilaginous membranes that could stick to water dwelling mammals and birds

(Smolders et al, 1995b); furthermore, turions descend to the sediment more quickly than seeds (Renman, 1989a) potentially limiting the amount of time they could be openly exposed to herbivory. While actual accounts of seed predation exist (Smolders et al, 1995b), turion herbivory has not yet been proven.

1.3.3 Distribution

S. aloides is native to Eurasia and its historical distribution covers Sweden, Norway, Finland, Denmark, Ireland, Scotland, England, France, Switzerland, Belgium, Holland, Germany, Poland, Italy, Spain, Austria, Czechoslovakia, Hungary, Yugoslavia, Romania, Bulgaria, Turkey, Siberia and Russia (Cook and Urmi-Konig, 1983).Fossil records have established that *Stratiotes aloides L.* was present in England during the mid-eocene period, about 45 million years ago (Cook and Urmi-König, 1983).

In North America, the known distribution of this species is limited to southern Ontario, Canada, where it can be found growing in two natural ecosystems (Lake Seymour and the Black River) and three private ponds (Trent Hills, Bayfiield and Blackstock) in the province of Ontario. The most well established population of *S. aloides* was discovered in 2008 and exists within a section of the Trent River, part of the Trent-Severn Waterway (**Figure 5**). This population serves as the focus for the research presented in this thesis. The TSW is a system of lakes and rivers that connects lakes Huron and Ontario over a distance of 386 km and features 45 locks that support navigation across the system. The section of the Trent River that *S. aloides* has colonized is located in what is locally known as Seymour Lake. Since its arrival, *S. aloides* has established populations in over 200 ha of this water body. Despite its name, this portion of the system is more so a series of back bays and widened main river channels than a traditional lake. The maximum water depth is about 12 m and the main TSW channel follows the deepest parts of the river through Seymour Lake to allow for ease of boat transit. Apart from the boat channel, the average depths of the river run between 1.5-3 m.

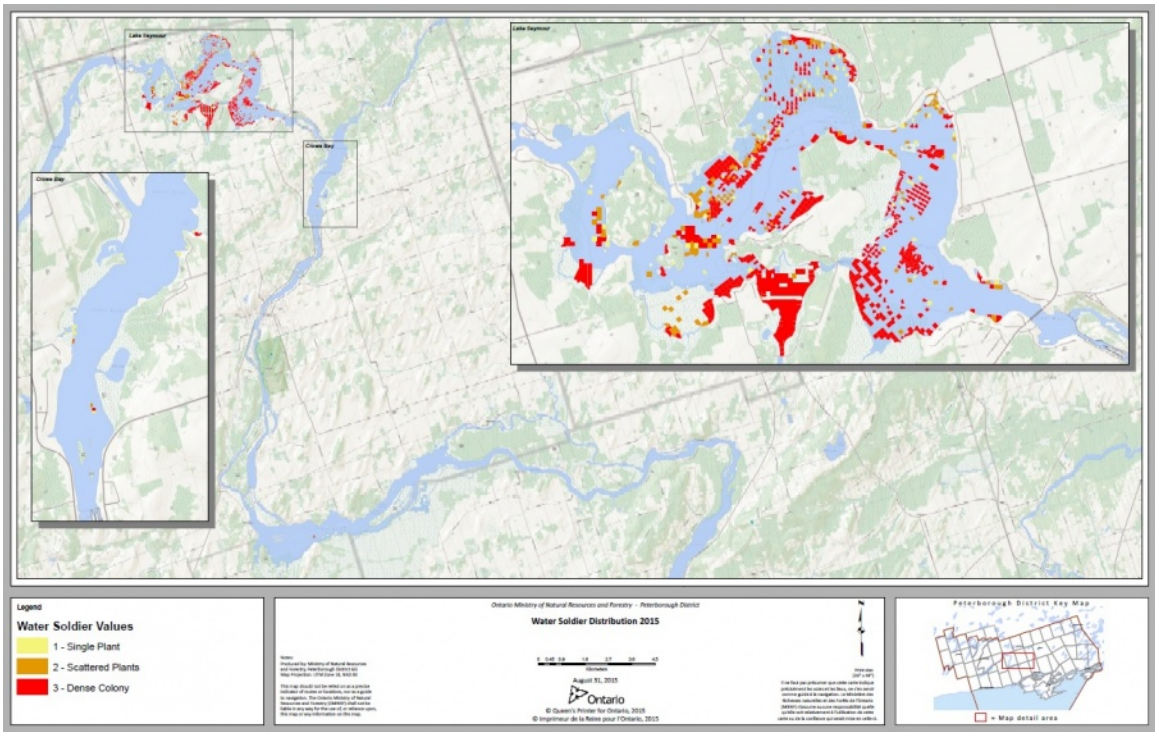


Figure 6: Map of Trent River showing distribution of the first North American *S. aloides* population in Lake Seymour and Crowe Bay and spread of plant down-river (OMNRF, 2015)

The surrounding shores of Seymour Lake are primarily heavily developed cottage and residential areas, with large sections of the shoreline replaced by concrete water breaks and barricades. Multiple resort, lodge, and fishing camp operations are present within the area. The naturalized shoreline, the majority of which can be found amongst the secluded back bays, is a mix of dense *Typha latifolia* (Common cattail) stands and local shrubby and herbaceous wetland plant species.

Aquatic plants colonize almost the entire area of the Seymour Lake waterway. Common species include plants like *Vallisneria americana* (Water celery), *Ceratophyllum demersum* (Coontail), *Elodea canadensis* (Canada Waterweed), *Myriophyllum spicatum* (Eurasian Water-Milfoil) and *Potamogeton crispus* (Curly-Leaved Pondweed). Despite the presence of established plant communities before the arrival of *S. aloides*, the abundance of both native and invasive species has declined in response to the spread of this macrophyte

More recently (2015), *S. aloides* was found growing in a second natural ecosystem on the Black River, which connects to the southern shore of Lake Simcoe. This population was estimated at ~1 ha and an attempt at quarantine was made by surrounding the population with anchored turbidity curtains, to prevent further spread. The majority of the plants within the area were manually removed. This species has also been identified as growing within three private ponds in the province (which is permitted), but no direct transmission route between these locations and public water systems exists.

The Michigan Department of Agriculture and Rural Development (MDARD) used plant hardiness zones, annual precipitation groups and Köppen-Geiger climate classes (vegetation biomes) to predict the potential habitat suitability of North America for *S. aloides* growth (MDARD, 2015). The map, provided in **Figure 6**, shows that 78% of the total area could support the existence of *S. aloides* populations; including almost 100% suitability in Ontario (MDARD, 2015). Although this model did not include growth factors specific to aquatic plants (including light limitation and nutrients), variations in waterway morphology or competition with other prodigious invasive species in the environment (i.e. *Hydrilla verticillata* (Hydrilla) and *Eichhornia crassipes* (Water hyacinth)), it stands as a good predictor of the invasive potential of *S. aloides* in North America.

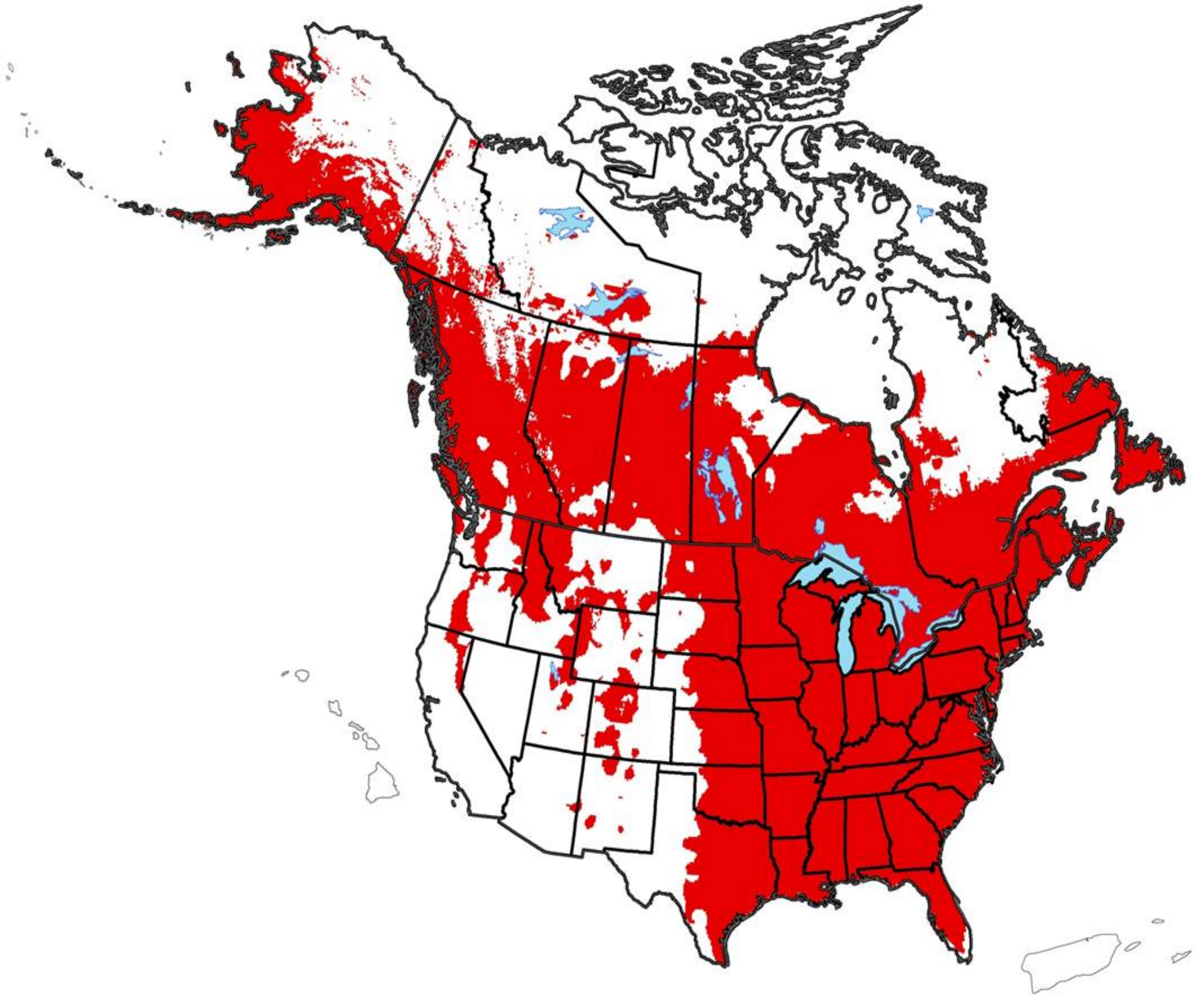


Figure 7: MDARD climate based, predicted habitat suitability map for *S. aloides* in North America. Based on plant hardiness, annual precipitation and Köppen-Geiger climate classes, 78% of the total area is suitable for *S. aloides* colonization including almost 100% of Ontario waters (MDARD, 2015, used with permission from Michigan Department of Agriculture and Rural Development, 2016)

1.3.4 Habitat Requirements

S. aloides is well suited to growing in Ontario as it is exposed to similar environmental and climatic conditions within parts of its native range. Unlike tropically sourced invasive aquatic macrophytes like *Eichhornia crassipes* and *Pistia Stratiotes* (Water lettuce), this plant can resist the cold and freezing temperatures that can sometimes be relied upon to kill invading organisms. Studies on populations of *S. aloides* in northern Sweden have documented offset production continuing throughout the winter months and under ice thicknesses of over 70 cm (Renman, 1989a).

S. aloides plants prefer backwater and sheltered areas (i.e. lake bays, canals and drainage ditches) and are susceptible to displacement by high rates of water flow (Cook and Urmi-König, 1983). The robust morphological structure of and high quantity of biomass produced from a single *S. aloides* plant, coupled with its weak root structures mean that swiftly flowing waters can easily dislodge plants from existing colonies and prevent the establishment of populations in those areas (Cook and Urmi-König, 1983; Madsen et al, 2001; Tor Nielson and Borum, 2008). Although bodies of water with high rates of flow can limit the success of this species, the high biomass and density of plants within established colonies of *S. aloides* can actually limit moderate water flow throughout a system (De Geus-Kruyt and Segal, 1973; Strzalek and Koperski, 2009).

S. aloides grows best in moderately alkaline waters and requires relatively high nutrient concentrations to support its growth (Cook and Urmi-König, 1983). Potassium, calcium and sodium uptake by these plants is especially high and studies have shown *S. aloides* as responsible for significantly lowering the concentration of these elements in water bodies, creating situations of limited nutrient availability for competing organisms

(Mulderij et al, 2005, 2009). In its native range, *S. aloides* has been susceptible to changing hydrological conditions and iron deficits leading to hyper-eutrophication (Bakker et al, 2016; Roelofs, 1991; Smolders et al, 2003). Sulphide and ammonium toxicity have been identified as factors responsible for sharp declines of *S. aloides* populations in some areas of its native distribution (Smolders et al, 2003).

S. aloides does demonstrate some degree of habitat elasticity, as it has been found to tolerate a range of pH and temperature values. *S. aloides* has been observed growing at pH levels ranging from 4.5-8.5 and lake trophic classifications ranging from oligotrophic to eutrophic (Cook and Urmi-König, 1983; Tor Nielson and Borum, 2008). Within the northern reaches of its native range, this species has been observed actively photosynthesizing and producing offsets under ice thicknesses of 70 cm (Renman, 1989b), showing some degree of cold tolerance. Despite this, low seasonal temperatures have been cited as a possible reason for the decline of sexual reproduction and the disappearance of male plants amongst *S. aloides* populations in the United Kingdom (Forbes, 2000). Although not conclusive, evidence suggests that *S. aloides* plants could require long, warm summers to trigger flower and seed production and that male plants are more susceptible to cold temperatures than female plants (Forbes, 2000).

Direct links have been made between light availability and *S. aloides* growth. Light is thought to be largely responsible for dictating biomass production, rosette buoyancy and range expansion of this species within its native habitat (Harpenslager et al, 2015). Experiments focused on the restoration of this species within its native range by Veen et al (2013) used enclosures to limit herbivory by grazing bird species. Incidentally, *S. aloides* growth increased by 60% within the enclosed areas as a result of the enclosure materiel reducing turbidity and increasing light availability to the plants (Veen et al, 2013). Harpenslager et al (2015) found that light availability was the most important factor in determining *S. aloides* growth, their ability to rapidly create dense surface mats and to colonize new habitat (Harpernslager et al, 2015). This relationship, however, has not been thoroughly quantified within its native range or in Ontario in terms of what depth range or light intensity values are needed to promote or suppress *S. aloides* success.

1.3.6 Competitive Traits

This species exhibits several competitive strategies that, compared to local plants, have allowed it to outcompete established ecosystems within the Trent River. These traits result in the ability to produce large quantities of biomass at a high rate, while suppressing the surrounding community. The ability of this species to perform vertical shifts within the water column during peak times in the growing season not only increases light availability, but the formation of dense emergent mats that effectively shade out other submersed macrophytes trying to grow underneath. *S. aloides* colonization has been shown to decrease the diversity and species richness of existing macrophytes both in Ontario (Canning, 2012) and in its native range (Sugier et al, 2010).

S. aloides plants have been shown to retain up to 60% of their midsummer vegetative biomass while they overwinter. This has been suggested as a method for plants to conserve potentially growth-limiting nutrients for new spring growth (Renman, 1989a). Additionally, compared to aquatic plants that need to start their life cycles in the spring from seed or rhizomes, *S. aloides* rosettes can immediately start allocating nutrients for reproduction, without the need for intensive vegetative growth first. With a high nutrient demand and efficient uptake ability, *S. aloides* plants can be so efficient at removing nutrients from their environment that they have been shown to restrict the growth rates of competing plant species (Kufel et al, 2010). Applied over the large area created by monospecific stands, this creates the opportunity for increased light and nutrient availability within those systems for *S. aloides* expansion caused by a reduction in competitor plants and phytoplankton within the water column (Kufel et al, 2010).

This species has also been found to possess allelopathic capabilities and can inhibit the growth of neighboring autotrophs (Cook and Urmi-Konig, 1983; Mulderij et al, 2005, 2009). In laboratory and *in-situ* incubation trials, the density, growth rate and biomass of surrounding phytoplankton species were reduced following contact with *S. aloides* plants and exudates (Mulderij et al, 2005). In field experiments, *S. aloides* was found to be the only plant capable of growing within dense mats of phytoplankton and it was hypothesized that production of allelopathic chemicals was the reason why *S. aloides* could inhibit phytoplankton growth and successfully compete within that environment (Mulderij et al, 2009). The inhibition of competing autotroph growth through allelopathic compounds could be another means by which *S. aloides* can facilitate the uptake of more resources, suppression of competing species and expansion of its population.

1.3.7 Potential Impacts

Where present, *S. aloides* is known to dominate its ecosystem with fast biomass production and the formation of expansive clonal genets (Cook and Urmi-Konig, 1983; De Geus-Kruyt and Segal, 1973; Strzalek and Koperski, 2009). It is almost always found in nearly monospecific stands of much lower plant diversity than in surrounding vegetation community types (Canning, 2012; Sugier et al, 2010). *S. aloides* has the potential to impact ecological, economic and social factors within the Trent-Severn Waterway, but the extent of those impacts in Ontario has not been studied.

Negative ecological impacts are likely to include the gradual colonization of suitable open water areas within the system, coupled with an overall drop in the diversity in existing aquatic plant communities. The possibility exists for this shift in vegetation type to cause a cascade of changes to the: phytoplankton, zooplankton, fish, macroinvertebrate, mammal and bird communities that have existing structural and function relationships to the native ecosystems in these areas.

Where present, *S. aloides* almost always is the dominant macrophyte species, establishing nearly 100% surface area cover and capable of outcompeting all other existing vegetation (De Geus-Kruyt and Segal, 1973; Mulderij, 2005; Renman 1989b). In small, enclosed water bodies, the sheer abundance of vegetation created by these plants has been shown to accumulate over time and initiate the transition from an aquatic to a terrestrial ecosystem (De Geus-Kruyt and Segal, 1973; Kufel et al, 2010; Strzalek and Koperski, 2009).

In its native distribution, the growth of *S. aloides* has sometimes been seen as a benefit to the aquatic community. The dense mat and root systems formed by the plant offer habitat to a number of fish and macroinvertebrate species. *S. aloides* is reported to support diverse communities of phytomacrofuana (macroinvertebrates associated with aquatic plants), with particularly high densities of: Chironomidae, Cladocera, Oligochaeta (Linhart, 1999; Strzalek and Koperski, 2009). Strzalek and Koperski (2009) found that *S. aloides* beds held significantly higher concentrations of zooplankton than other sampled environments in the littoral and pelagic zones in their study lake and that zooplankton abundance and diversity was dependent upon the presence of this species (Strzalek and Koperski, 2009). In Europe, the endangered species *Aeshna viridis* (green-hawker dragonfly), has been identified as a *S. aloides* specialist that is almost exclusively found in *S. aloides* stands. The decline of *A.viridis* has been linked to the disappearance of *S. aloides* across its native range (Rantala et al, 2004). Fish commonly found using these stands for food and habitat include: Northern pike (*Esox Lucius*), European perch (*Perca fluviatilis*), Common rudd (*Scardinius erythrophtalmus*) and the Crucian carp (*Carassius carassius*) (Strzalek and Koperski, 2009).

There is anecdotal evidence that *S. aloides* may interact positively with other high priority invasive species in the TSW such as *Neogobius melanostomus* (Round goby), *Orconectes rusticus* (Rusty crayfish) and *Dreissena polymorpha* (Zebra mussel). The unique morphology and life history traits of this plant appear to serve as valuable overwintering habitat for these species. (Canning, pers. Obs).

The economic impacts associated with *S. aloides* include the costs of monitoring, management, and public education and outreach as the species introduction progresses. Management costs alone for *S. aloides* in Ontario are estimated at between $1500 and $48 000 per hectare (OMNRF, 2013). Economic impacts of an invasive aquatic species within an area can also be measured through drops in recreational, tourism and property tax revenue and a decrease in real estate values closest to the infestation (Horsch and Lewis, 2009).

The social and economic impacts of the *S. aloides* infestation will be most severely felt by the residents of, and visitors to, the Trent River area. A designated national historical site, the Trent Severn System is one of Canada’s most widely used waterways as it connects lakes Huron and Ontario while winding through popular vacation and tourism centers and densely populated areas (Parks Canada, 2016). Communities along the banks of this waterway rely on it for drinking water, recreation and livelihood. The annual financial contributions of the Trent Severn Waterway to the economy of Ontario are formidable: businesses and residential properties lining the TSW generate over $240 million in property taxes; the recreational fishery contributes $300 million, while residents and tourists represent more than 1 billion in economic activity (Panel on the Future of the Trent Severn Waterway, 2008). As a primary conduit through the Great Lakes system, it is an essential artery for shipping of agricultural and industrial commodities in Canada. Extensive growth of the plant can interfere with navigation and boat movement, fishing and hunting opportunities and water-based recreational activities. The potential negative impacts of *S. aloides* infestation in Ontario are considerable. The present research is designed to provide valuable information to inform management and treatment plans that could reduce the impact of this aggressive invasive aquatic plant on Ontario waterways.

1.4 Goals and Objectives

The primary purposes of this research were to generate key information on *S. aloides* in regards to its biological response to growth limiting factors in the Trent River, to document the response of *S. aloides* and local plant communities following the administration of two potential treatment methods (chemical control and manual raking) and to inform the creation of management plans for effective eradication of *S. aloides*. The specific objectives of this study were to:

1) Determine the relationship between light intensity and *S. aloides* propagule response in determining successful plant growth and depth based distribution by:
    a. Describing the role of light as a primary limiting growth factor for *S. aloides* in the Trent River
    b. Determining the light requirements for active photosynthesis in *S. aloides* clonal offsets and turion germination
    c. Predicting the maximum depth(s) of colonization and persistence for *S. aloides* within the Trent River
2) Test two management techniques (chemical control and manual raking) for *S. aloides* based upon biological knowledge of this species in Ontario, current management goals and local site conditions by:
    a. Quantitatively testing selected treatment methods to determine their effect on *S. aloides* populations
    b. Monitoring the effects of the *S. aloides* treatment methods used on the surrounding macrophyte community.

CHAPTER 2: The influence of light for *S. aloides* growth in Ontario

2.1 Introduction

When managing invasive plant populations, especially new introductions, the question of what dictates successful growth of the target species becomes critical for understanding the potential for it to colonize new habitats and cause harm to local ecosystems (Simberloff, 2003). The mechanisms behind the success of aquatic plants and the factors that can potentially limit the growth of macrophytes have been well studied in the literature. This includes a wide range of variables such as: temperature (Dale, 1986), water movement (Riis and Biggs, 2001), herbivory (Lodge, 1991), nutrient availability (Barko et al, 1991), competition (McCreary, 1991) and lake morphology (Caffrey et al 2007; Schutten and Davy, 2000).

Most commonly, however, light availability has been reported as the most important factor for successful aquatic plant growth (Barko et al, 1982; Barko et al, 1991; Barko and Smart, 1981; Bornette and Puijalon, 2011; Chambers and Kalff, 1985; Dale, 1986; Dar et al, 2014; Dennison, 1987; Dennison et al, 1993; Kufel et al, 2010; Lacoul and Freedman, 2006; Lodge, 1991; McCreary, 1991; Middleboe and Markager, 1997; Riis and Biggs, 2001; Sand-Jensen, 1989; Schutten and Davy, 2000; Schwarz et al, 2000; Zimmerman et al, 1994; Van den Berg et al, 2003). In light limited ecosystems, aquatic plant communities tend to shift and show dominance towards canopy forming macrophytes that can maximize light absorption and minimize light competition from other species (Kufel et al, 2010; Pieczyriska et al, 1988). This trend was exemplified in Ontario, where once introduced, large floating canopies of *S. aloides* plants quickly replaced submerged macrophyte communities as the dominant plant type (Canning, 2012).

Within its native range, *S. aloides* has been acknowledged to be present in and have greater success in deeper water habitats compared to competing aquatic plants (Veen et al, 2013). Harpenslager et al (2015) studied the role of photosynthetically available radiation (PAR) on photosynthesis and biomass production in *S. aloides* by exposing *S. aloides* rosettes to high, medium and low light intensities (130, 50 and 15 $\mu mol/m^{-2}/s^{-1}$ respectively). They found that PAR was the most important factor for determining *S. aloides* growth and plants grown under medium and low light intensities produced significantly less biomass and clonal offsets than plants grown under high intensity light. Plants under high intensity lights were able to triple their biomass within only four months of exposure (Harpenslager et al, 2015).

Most importantly, Harpenslager et al (2015) found that only plants subject to high light intensity were able to develop into a fully buoyant, emergent canopy layer. In the case of *S. aloides*, they found that rosette buoyancy enabled plants to form dense, light suppressing canopies and promoted fast colonization. Medium and low light intensity exposed plants, however, never became fully buoyant and were characterized by low biomass production and colonization potential (Harpenslager et al, 2015). Presumably, plants that are able to become buoyant can reduce or eliminate completion from other plants by inducing light limitation below the canopy and are much more likely to be responsible for fast and widespread colonization of a water body. Despite its link to biomass production and the facilitation of *S. aloides* colonization, the role of light in determining the growth success and invasive potential of *S. aloides* has not been well studied in Ontario.

This chapter summarizes and discusses light as a key limiting factor that may both promote and inhibit *S. aloides* growth in the Trent Severn Waterway. Specifically, it will address the effect of changes in light intensity on i) rates of photosynthesis in *S. aloides* offsets and ii) germination abilities of *S. aloides* turions. Based on these findings, the depth of maximum colonization (Z_c) and the invasive potential for *S. aloides* within the Trent River will be predicted.

2.2 Factors Influencing *S. aloides* Growth

2.2.1 Temperature

Climate can play a large role in determining the distribution of a macrophyte species (Barko and Smart, 1981), and temperature is sometimes viewed as the most effective way of controlling an invasive aquatic plant species or predicting its ability to colonize new habitats on a north-south gradient (Owens and Madsen, 1995). Drawdown of water levels in controlled systems to expose the propagules of invasive macrophytes to extreme temperatures has been shown to be an effective treatment method for managing nuisance plant growth (Capers et al, 2005; Dugdale et al, 2012; Helton and Hartmann, 1995; Richardson, 2008). Exposure to freezing temperatures has also been shown to act as a natural control to mitigate the spread of tropical invasive plants into more northerly locales (Barko and Smart, 1981; Dukes and Mooney, 1999; Rahel et al, 2008). Aquatic plants grown in temperature ranges outside of the tolerable limits for that species have been found to alter their morphological growth structure and lower overall biomass production (Barko and Smart, 1981).

Temperature plays a less important role when considering invasive *S. aloides* populations in Ontario. *S. aloides* has shown itself tolerant to the local winter temperatures with no depreciable effects on yearly plant abundance. *S. aloides* has also displayed its resilience to exposure to above average seasonal temperatures by ascending from the sediment to become an emergent plant and showing a preference for full exposure to light and summer temperatures. To lessen the negative effects of high temperature on plant growth, the morphology of emergent *S. aloides* plants changes to include stomata and waxy leaves and cuticles (Tor Neilson and Borum, 2008), which are typical features used to prevent desiccation by plants in dry, hot environments.

The observed trends of temperature related die-offs for some invasive plant species moving outside of their historical geographic range are not as applicable to *S. aloides* because the climate it is exposed to in Ontario is similar to that in parts of its native range. Literature on this species from its native distribution often describes the presence of healthy *S. aloides* tissue and offset production when sampled through the ice (Cook and Urmi-Konig, 1983; Renman, 1989a, 1989b). Not only can this species endure periods of sub-zero temperatures (Renman, 1989a), it might even rely on them to decimate the standing biomass of less robust macrophytes in the water column to provide a competitive advantage in the spring (Barko and Smart, 1981). *S. aloides* plants can retain up to 60% of their peak midsummer biomass while overwintering as rosettes (Renman, 1989a). As a competitive life history mechanism, when native plant growth resumes in earnest in the spring, *S. aloides* already has the physiological and morphological structures needed to begin propagule production, without the need for an initial period of tissue growth.

2.2.2 Water Movement

The relationship between macrophyte distribution and water movement is extremely complex and multifaceted. Current and wave action can as easily facilitate macrophyte presence as eliminate it. River dwelling populations of aquatic plants are particularly susceptible to influence by water movement, given the constant force of water moving through those types of systems. Water movement can limit or change macrophyte distribution and colonization in a number of ways including: increasing light attenuation in the water column, dislodging or physically breaking plants, decreasing epiphytic growth, changing the level of photosynthetic activity, directing nutrient and sediment accumulation and causing substrate erosion (Bal and Miere, 2009; Franklin et al, 2008; Madsen et al, 2001).

The force of moving water acting on a macrophyte can be equated as a function of water velocity, biomass and plant growth form (Dawson and Robinson, 1984; Madsen et al, 2001). This value differs from species to species, largely based on frequency of stem branching and leaf presence (Bal and Miere, 2009; Franklin et al, 2008; Madsen et al, 2001). Dawson and Robinson (1984) calculated drag force values for a number of aquatic species, including some that are present in the Trent River and found that drag force values increased in species with leaf bracts, whorls and multiple meristems versus species with basal growth and long, ribbon-like leaves.

In the context of the Trent River, water movement may be most relevant to macrophyte distribution in areas immediately adjacent to the current that flows through the main river and navigational channel (which comprises a very small portion of available habitat in the system). In these areas, high rates of flow velocity can easily make the establishment of populations of plants with high water resistance and low rooting strength, like *S. aloides,* impossible (Franklin et al, 2008; Haslam, 1978). River currents can also move individual plants away from high velocity areas and congregate them in the nearest protected bays or eddies where the force of water flow is lower (Nilsson et al, 1991). The effects of water movement can certainly restrict the growth of *S. aloides* in high flow areas of the system, but overall, it would appear that water movement is likely to have a neutral or even slightly positive effect on the colonization and distribution of *S. aloides* in the Trent River. This species can use its photosynthetic abilities to not only reduce the impacts of current based sedimentation, but it can outcompete other macrophytes in the river if it does get dislodged and relocated through the force of water movement. Current can also funnel *S. aloides* plants into adjacent areas and inadvertently increase the rates at which this species accumulates its population biomass. Propagule distribution by hydrochory remains one of the most important means of long distance dispersal for *S. aloides* and the water movement along the Trent River helps to increase the effectiveness of this life history mechanism (Sarneel, 2013; Smolders et al, 1995a).

2.2.3 Herbivory

In certain situations, herbivory can be a critical factor limiting submersed plant growth. Van Donk et al (1994) studied the impact of increased *Fulica americana* (American coot) populations on the abundance of aquatic vegetation in Lake Zwemlust, Netherlands. They found that this one species of waterfowl alone was able to consume up to two kilograms of dry plant weight per day and represented an intensive disturbance to submerged vegetation in that water body. Macrophyte community composition was even seen to change as a direct result of the *F. americana* herbivory (Van Donk et al, 1994). Fish can also play a significant role through plant grazing. *Ctenopharyngodon idella* (grass carp) not only ingest aquatic plant matter for food, but their aggressive foraging activities can increase water turbidity and dislodge surrounding vegetation that is important for other organisms, but not utilized by the fish. The exclusion of this species from highly disturbed and sensitive areas of a water body has been directly related to aquatic plant community recovery (Lougheed et al, 2004; McKnight and Hepp, 1995; Wilcox and Whillans, 1999).

Despite noted occurrences of severe aquatic plant community suppression due to vegetation grazing, this cannot be identified as a major factor for limiting plant growth of *S. aloides* in the Trent River or its native range. Accounts of *S. aloides* herbivory exist in the literature, but mainly by macroinvertebrate species (Linhart, 1999). There are no records of severe impacts on *S. aloides* growth from herbivory in its native range or in Ontario, but the high nitrogen content in its leaves has been suggested as an attractant to herbivores (Cook and Urmi-Konig, 1983; Linhart, 1999). Given the relatively recent introduction of this species to the Trent River, it could be that herbivores in that area are yet to recognize or utilize this species as a food source, but herbivory could rise as the duration of the invasion increases. For example, *Branta canadensis* (Canada geese) have been observed feeding on the leaves of S. aloides rosettes (pers. obs.)

2.2.4 Nutrient Availability

Nutrient availability is vital to submersed plant growth (Hutchinson, 1975; Sculthorpe, 1967). Both excessive and insufficient concentrations of nutrients can negatively impact macrophyte success. The most essential nutrients for aquatic plant growth are nitrogen (N) and phosphorous (P) (Mebane et al, 2013; Moss et al, 2013), but potassium (K), magnesium (Mg), calcium (Ca), sodium (Na), iron (Fe) and manganese (Mn) have also been identified as important for aquatic vegetation (Barko and Smart, 1986).

Without conducting a full analysis on the nutrient content of *S. aloides* tissues, sediments and water samples from the Trent River, the extent to which nutrient availability could limit *S. aloides* growth and establishment must be hypothesized. Apart from the main navigation channel, almost the entire extent of available habitat on the Trent River is colonized by aquatic vegetation. If nutrients were limiting in this system, the yearly growth of aquatic macrophytes would not be so consistent or so widesprea*d. S. aloides* is frequently described as showing a preference for and only successfully growing in nutrient rich waters (Kufel et al, 2010; Sculthorpe, 1967; Smolders et al, 2003; Strzalek and Koperski, 2009; Suutari et al, 2009; Tor Nielson and Borum, 2008). The water bodies in Ontario where *S. aloides* is currently present (including the Trent River) are typically classified as nutrient rich and eutrophic waters.

This plant utilizes sediments and water for nutrient sources (Brammer, 1979; Mulderij et al, 2005; Sugier et al, 2010) and its ability to uptake any available nutrients in an ecosystem is so great that it has been shown to inhibit the growth of surrounding macrophytes through resource denial (Kufel et al, 2010; Mulderij et al, 2005, 2009). Nutrients, specifically dissolved inorganic carbon, can limit photosynthetic activity especially in environments of high light intensity to which emergent *S. aloides* rosettes are exposed (Madsen and Sand-Jensen, 1994). To counter this, the floating *S. aloides* leaves develop stomata to allow for increased atmospheric carbon intake that enables the plant to raise its light saturation point (Tor Nielson and Borum, 2008).

Excess nutrient availability (hyper-eutrophication) has, however, been identified as a major factor in the decline of this species within its native range (Smolders et al, 1996). Phosphorous and sulphate loading in the Netherlands have increased to such a degree that hyper-eutrophic adapted species of non-rooting macrophytes and phytoplankton are the only types of organisms able to live in such conditions and have outcompeted *S. aloides* in terms of community dominance (Smolders et al, 1996). In such eutrophic conditions, *S. aloides* is susceptible to sulphite and ammonium toxicity (Smolders et al, 2003). The increasing rate of nutrient loading in Ontario waters could make hyper-eutrophication a limiting factor in the long-term survival of this species in some areas.

2.2.5 Plant Competition

From the perspective of *S. aloides* ramets expanding their population size and immediate distribution, competition from surrounding macrophytes may not be a significant factor. In its native distribution, this plant has been rarely found co-existing with a diverse array of other macrophyte species (Canning, 2012; Kufel et al, 2010) and typically dominates the local plant community (De Geus-Kruyt and Segal, 1973; Strzalek and Koperski, 2009). *S. aloides* can grow until 100% coverage of surface water in small lakes has been established and it has even prompted the conversion of aquatic ecosystems into terrestrial environments (De Geus-Kruyt and Segal, 1973; Kufel et al, 2010). These occurrences can only take place in a system where the growth of *S. aloides* remains unchallenged by the local plant community. Even when growing in areas surrounded by dense vegetation beds, *S. aloides* has proven itself an able competitor. In the Morava River, Slovakia, *S. aloides* populations showed a 40% increase in distribution over two years to the detriment of previously dominant *Hydrocharis moresus-ranae* (European frogbit) and *Lemna trsicula* (star duckweed) communities (Otahelova and Banasova, 1997).

S. aloides has developed unique life history strategies to counter the potential negative effects of plant competition. Rapid biomass production can shade out macrophytes growing under the canopy of emergent *S. aloides* rosettes and overwintering tissue retention can provide a resource advantage to the plants at the onset of the growing season (De Geus-Kruyt and Segal, 1973; Otahelova and Banasova, 1997). High rates of nutrient uptake help to inhibit the growth of surrounding plants, while increasing their own energy storage (Kufel et al, 2010). Finally, low light tolerance enables *S. aloides* to survive at depths below the capabilities of local plants and increases the recruitment potential of its propagules (Harpenslager et al, 2015; Veen et al, 2013).

2.2.6 Light Availability

Although a plant may have access to sufficient nutrient resources, exist in a climate within its preferred range, be exposed to stimulating rates of water movement and be free from disturbances, without adequate light availability, that plant cannot grow. Light is an essential requirement for all aquatic plants and is the most important factor for photosynthetic production (Binzer et al, 2006). This fact gives photosynthetically available radiation (PAR) distinct importance when considered against other macrophyte limiting factors (Zimmerman et al, 1994). The attenuation of light throughout the water column can restrict the depths at which plants grow and can render certain species completely unable to survive (Chambers and Kalff, 1985). Light intensity and availability are widely accepted as the most significant factors influencing macrophyte growth (Barko et al, 1982; Barko et al, 1991; Barko and Smart, 1981; Bornette and Puijalon, 2011; Chambers and Kalff, 1985; Dale, 1986; Dar et al, 2014; Dennison, 1987; Dennison et al, 1993; Kufel et al, 2010; Lodge, 1991; McCreary, 1991; Middleboe and Markager, 1997; Riis and Biggs, 2001; Sand-Jensen, 1989; Schutten and Davy, 2000; Schwarz et al, 2000; Zimmerman et al, 1994; Van den Berg et al, 2003).

In systems limited by light availability, plant communities modify their composition to show a preference for macrophytes best suited to maximizing light absorption and tolerating low light (Kufel et al, 2010). This shift often manifests itself as an increase in the dominance of canopy-forming, monospecific, community-promoting species (Pieczyriska et al, 1988), such as *S. aloides*. Given its importance as a limiting factor for macrophyte growth and spread, light was selected as a key variable of interest in this research in hopes of predicting *S. aloides* ability to colonize new territory, establishing larger populations and describing its invasive potential in the Trent River.

2.3 Materials and Methods

The effect of changes in light intensity to the growth of *S. aloides* propagules was studied by measuring net photosynthesis of offsets in the field and the germination response of turions under experimental conditions. Rates of photosynthesis for offsets were used to determine the light compensation point and to estimate a maximum depth of colonization for this species within the Trent River.

2.3.1 Net Offset Photosynthesis

The method used to measure net photosynthesis in young offsets was adapted from Bultemeier et al (2009) and Wetzel and Likens (1991). Incubations were conducted *in situ* on the Trent River (Lake Seymour) on July 25th and August 29th 2013. Healthy, green offsets were collected from mature *S. aloides* plants. Large variations in offset size and maturity were observed while collecting specimens for this experiment. To standardize the amount of plant material, all offsets were weighed and only offsets within a range of 15g +/-5g were used in the incubations. The weight range of samples collected in this study (15g +/-5g), was approximately one fifth the size of a typical young offset. Stolons were cut at the base of the offset and any roots present were removed. Plants were rinsed before incubation to remove aufwuchs and kept in a cooler until use.

The weighed offsets were put into 1-litre BOD bottles and were suspended at 1m intervals along a weighted rope (which also served as a depth measurement tool) starting at 0m and continuing down to the anchor point at 6m (the deepest portion of the waterway directly adjacent to *S. aloides* patches). At each interval, two light bottles (LB), one dark bottle (DB- these were created by covering BOD bottles in black electrical tape and aluminum foil) and one blank bottle (B) were attached to the rope (four bottles for every 1m-depth increment and four replicate arrays per sampling date) for three hours. On each incubation array, there were a total of 24 bottles spread between 0 and 6m. Care was taken to distribute each group of bottles on the anchor rope so as to limit shading and allow equal access to light. Four replicates of the offset suspensions arrays were used at each sampling date (July 25 and August 29th 2013).

Initial dissolved oxygen (DO) measurements using a YSI™ Model 550A dissolved oxygen meter were taken from river water the filled BOD bottles before placing one offset in each LB and DB bottle per interval. The blank bottles, which did not contain offsets, were used as a control to ascertain if there were variations in oxygen concentration due to phytoplankton without the presence of *S. aloides* plant tissue. Each string of bottles was allowed to incubate in the river for three hours, after which they were removed and the final dissolved oxygen measurements for each bottle were measured and water depth at each interval was recorded (YSI™ Model 550A dissolved oxygen meter). Offsets were dabbed with paper towels to remove excess water and the wet weight was recorded for each. Changes in the blank bottle oxygen concentration were subtracted from LB readings to adjust for non-offset oxygen production (Wetzel and Likens 1991, Spencer and Ksander 2001). Net photosynthetic rates were calculated using this equation derived from Bultemeier et al (2009) and Wetzel and Likens (1991):

$$\text{Net Rate } (\mu\text{g } O_2 / \text{g (fresh weight)/minute}) = \frac{[\text{Final DO } (\mu\text{g}) - \text{Initial DO}(\mu\text{g})]}{[\text{wet weight (g)} * \text{time (min)}]}$$

2.3.2 Turion Germination

Two separate rounds of turion germination experiments were carried out in a controlled micro-climate environmental chamber at Trent University throughout the fall and winter of 2013. The fully isolated 12’x15’ walk-in chamber was capable of simulating a wide range of temperature, humidity and light cycle/intensity parameters to fit the needs of this study. The first round started on October 8th and the second on December 31st, 2013 and both experiments ran for 40 days. Two tables were placed within the chamber and 15 32”x20”x5” plastic trays were placed on top. Two inches of Trent River sediment was spread over the bottom of each tray and the rest of the tray depth was filled with Trent River water. The trays were allowed to settle for 48 hours before commencement of the experiment.

To replicate actual light attenuation found on the Trent River, three intensity categories (low, medium and high) were pre-selected to test the effect of light intensity reduction on turion germination. To establish light categories for use in the experiment, light intensity measurements were taken at 0.5m intervals from the surface to the bottom of the Trent River on three occasions during the growing season. Measurements were taken on June 18th, July 25th and August 29th 2013 at midday under predominantly clear sky conditions. These measurements are presented in **Table 1**.

Table 1: Mean summer light intensity and % of surface light for the Trent River water column compiled from measurements taken on June 18th, July 25th and August 29th, 2013.

Depth (m)	Light Intensity ± SD (µmol $m^{-2}s^{-1}$)	% of Surface Light
0	1094 ± 154	100
0.5	731 ± 119	67
1	422 ± 54	39
1.5	241 ± 29	22
2	147 ± 40	13
2.5	98 ± 29	9
3	66 ± 8	6
3.5	39 ± 19	4
4	35 ± 2	3
4.5	23 ± 4	2
5	16 ± 3	1
5.5	8 ± 2	0.7
6	3.3 ± 1.8	0.3
6.5	1.9 ± 0.9	0.2

Based on these data, the average light intensity at depths of 1-1.5, 2.5-3, and 6-6.5m were calculated and used as the conditions for this portion of the study, based upon the assumption that these average levels of light intensity represent conditions that turions released from *S. aloides* plants on the Trent River would likely encounter. The high light intensity level (250 umol/m^2/sec or 22% of surface light) was selected to be similar to what turions naturally released in the shallowest areas of the river would encounter. The medium light intensity level (73 umol/m^2/sec or 6% of surface light) was selected to match what most released turions would be exposed to on the river because the largest and most dense populations of *S. aloides* are found around 3m depths. The low light intensity setting (3 umol/m^2/sec or 0.3% of surface light) represented the conditions faced by turions that descend into the deepest parts of the Trent River (6-7m) and near total dark conditions.

Each of the 15 trays was randomly assigned to one of these light intensity categories; therefore, the experimental design was comprised of three different light level categories, each with five replicate turion trays. To reduce the light intensity to match the designation of a particular tray, screening material was layered on top of the tray until the desired amount of attenuation was achieved (**Figure 8**). A portable Li-Cor 250A photometer was used to ensure equal light intensities within each of the three intensity categories. The screen was secured on the tray with heavy-duty, opaque tape that prevented ambient light from affecting the pre-selected exposure levels.

Turions were harvested from mature *S. aloides* plants during the late summer/fall production period and placed into trays within one day of harvest. Twelve turions were weighed and randomly placed in the water of each tray. There were five replicate trays used per light level and a total of 180 turions were used in each replicate of the experiment. Turions that did not germinate by the end of the experiment were extracted from the sediment using a fine sieve and weighed.

The experiments were run in a controlled climate chamber for 40 days using a 13:11 light:dark photoperiod. The daytime chamber temperature was set at 18^{o}C and nighttime at 10^{o}C, which aligns with conditions during early fall on the Trent River. The tray water level was monitored daily and was supplemented by river water additions to address evaporation, as needed. Comparison of the number of germinated turions between the trays was done through ANOVA in R.

Figure 8: Example of turion trays from the germination experiment carried out in the climate controlled environmental chamber. Black screening was used to regulate the amount of PAR reaching the turions. Pictured here are two medium light intensity trays with light attenuated to 73 umol/m^2/sec

2.3.3 Determination of Maximum Depth of *S. aloides* Colonization

The net rates of offset photosynthesis were plotted against water depth to create a productivity figure for *S. aloides* growing in the Trent River (**Figures 9-10)**. A linear model was used to fit a regression line to the data and the line equation was utilized to determine the depth at which net photosynthesis equals 0 (**Figures 14-15**). The calculated value represented the light compensation point (Z_{LC}), denoting the depth at which the rate of photosynthesis equals that of respiration for *S. aloides* (Glime, 2007; Spencer and

Ksander, 2001). Z_{LC} is commonly related to the lower depth distribution of aquatic plants (Z_C-maximum depth of colonization) and typically occurs at greater depths than Z_C (Hutchinson, 1975; McAllister, 1991; Van et al, 1976). Although plants may be present at depths in which they have established compensation (photosynthesis=respiration), they require higher light concentrations and positive rates of photosynthesis to actually produce biomass, reproduce, and persist (McAllister, 1991). The variance in depths at which an individual plant can sufficiently photosynthesize, grow, and persist compared to its compensation point is not known, but can be estimated. McAllister (1991) found that Z_C was typically 20% less than $Z_{LC.}$ This reduction was applied to Z_{LC} values measured in this study to estimate a final Z_C. Maximum depths of colonization have also been calculated using light attenuation throughout the water column and expressed as percentages of total surface light (Caffrey et al, 2007; Canfield et al, 1985). Macrophytes have been found to persist at maximum depths where approximately 11-0.75% of surface light is present (Caffrey et al, 2007; Canfield et al, 1985; Hutchinson, 1975). These estimates, however, were found to be highly subjective based upon plant species and lake morphology and it was concluded that Z_C determinations based upon actual light meter and photosynthesis measurements could be more accurate (Caffrey et al, 2007). As such, the 20% rule as established by McAllister (1991) was used in this study.

2.4 Results

2.4.1 Net Offset Photosynthesis

Rates of net offset photosynthesis for each sample period (July 25th and August 29th 2013) were negatively correlated to water depth (**Figures 9-10**). Photosynthesis from offsets declined sharply after 2m and stopped at ~5m water depth in both sampling periods. Rates of respiration exceeded those of photosynthesis in the LB's at the 5 and 6m intervals for both incubation dates. **Figures 9** and **10** resemble each other quite closely below depth=2m, showing steady declines in photosynthetic rates. This suggests that light attenuation was fairly consistent throughout the water column between the incubation periods. In the majority of the blank BOD bottles, DO concentrations increased and in all of the dark bottles, the DO concentrations decreased as anticipated (Appendix 2).

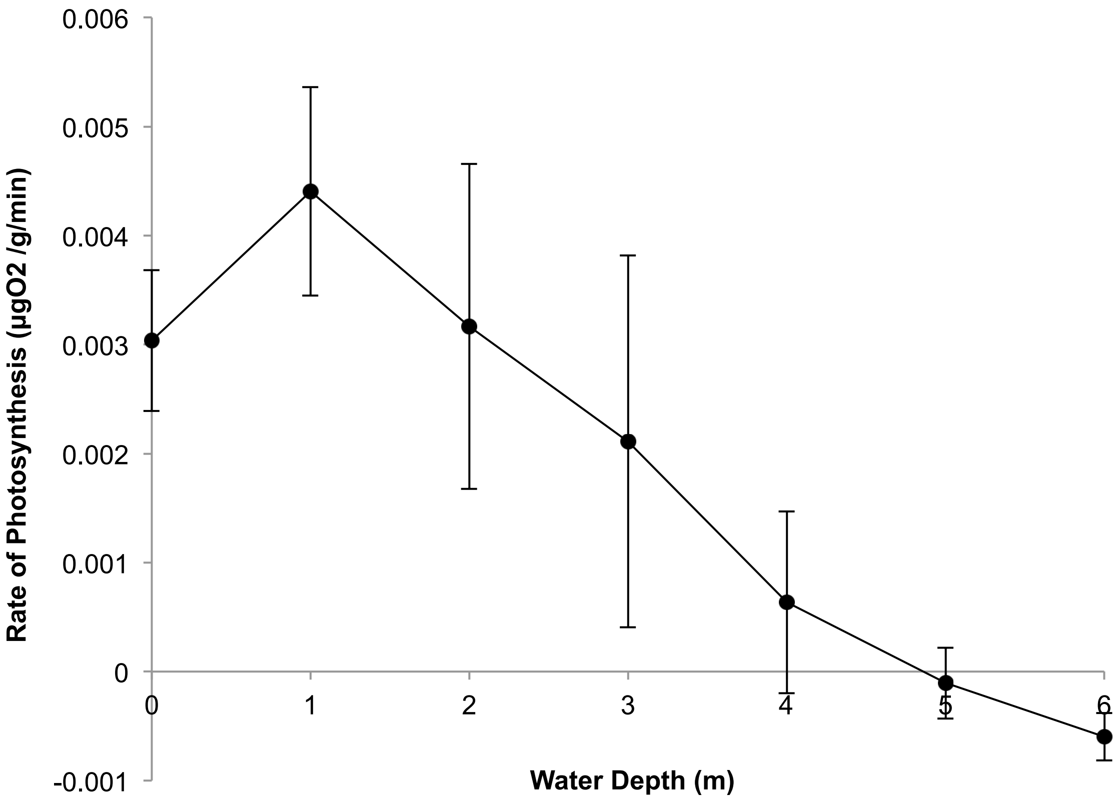


Figure 9: Rates of *S. aloides* offset photosynthesis related to water depth. Values were calculated by measuring changes in dissolved oxygen concentrations from offset incubations conducted *in situ* on the Trent River on July 25th, 2013. The incubation duration was three hours and was carried out under full-sun to partly-cloudy conditions using 15g ±5g offsets (n=8)

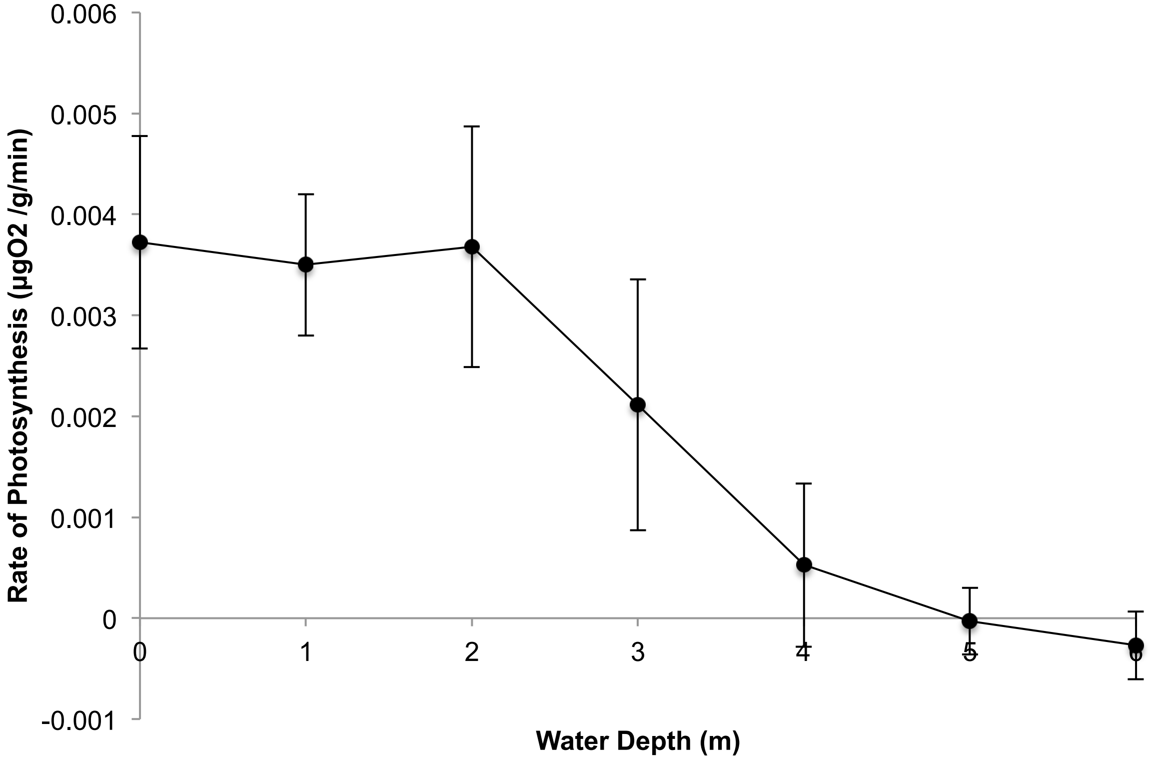


Figure 10: Rates of *S. aloides* offset photosynthesis related to water depth. Values were calculated by measuring changes in dissolved oxygen concentrations from offset incubations conducted *in situ* on the Trent River on August 29th, 2013. The incubation duration was three hours and was carried out under full-sun to partly-cloudy conditions using 15g ±5g offsets (n=8)

2.4.2 Turion Germination

Turion germination occurred in all three light intensity categories, although significantly more turions germinated in the high light intensity trays compared to the low intensity exposures ($p < 0.05$) in both rounds of the experiment (**Figure 12**). Data from the two experimental rounds were combined and results are reported below.

Turion germination advanced to different degrees depending on light intensity level. Turions were considered germinated when the bud scales opened and leaves began to expand beyond the confines of the propagule (**Figure 11**). In some cases, germination continued beyond this stage to open fully and form miniature rosettes with early stages of root development. In the high and medium light intensity trays, 40-43% of all germinated turions formed rosettes, while this was not observed in any of the low light intensity trays. Turions were weighed before the experiments and were found to have an average weight of 0.46g ± 0.06 (n=180). There was no relationship between turion weight and germination success at any light level ($p>0.05$) therefore turion weight was determined not be a factor.

Throughout the duration of the experiments, the trays required periodical removal of algal growth and non-target macrophytes introduced with the river sediment. The macrophyte species removed were *L. triscula* and *C. demersum* (coontail).

Figure 11: Example of germinated turion from the full light exposure trays (250 umol/m^2/sec) during the first round of the turion germination experiments. Bud scales open to allow rosette leaves to expand beyond the confines of the turion

An unexpected phenomenon was encountered during both rounds of the turion germination experiment, but was especially pronounced during the initial round of germinations. At the end of the experimental period when the turions were retrieved, it appeared that a certain portion of the turions that did not germinate had decomposed within the 40-day period and were no longer visible. Once this trend was recognized, sediment within the trays was sieved to verify these findings. Average rates of turion recovery in round 1 were as low as 57% and turion decomposition only occurred in the full and medium light intensity trays. In round 2, only one turion out of 180 was not recovered: it decomposed in one of the medium light intensity trays. No turion decomposition was observed in the low light trays in either round. Rates of average turion recovery can be seen in **Figure 13**.

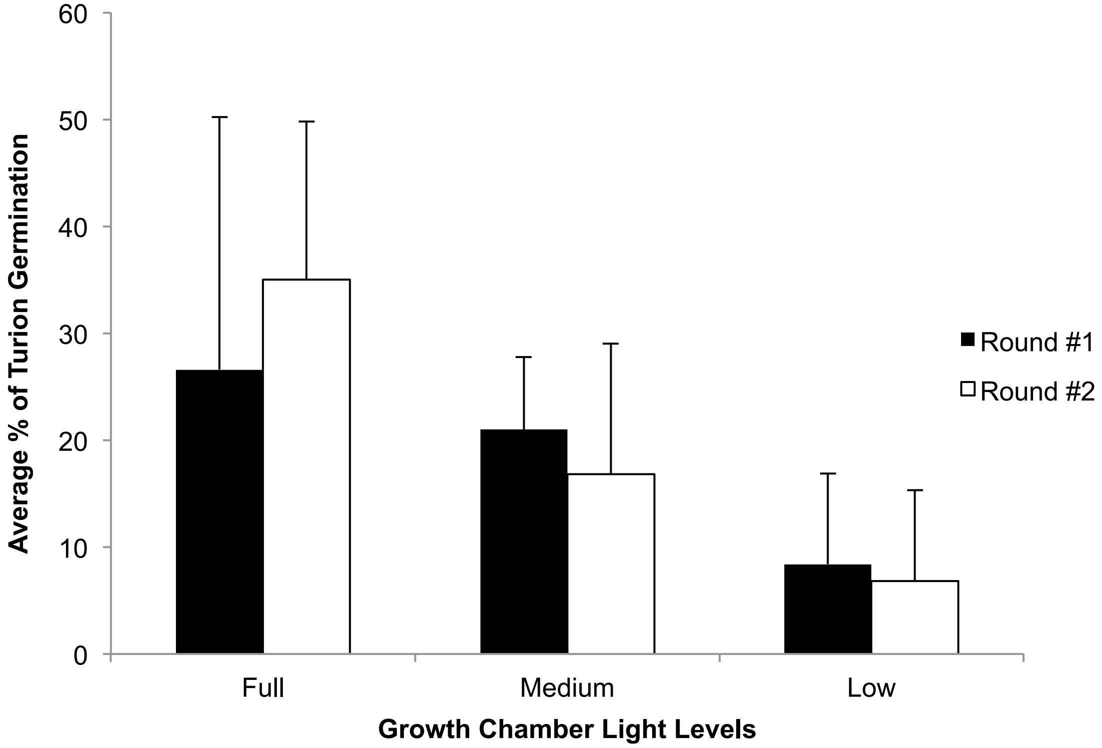


Figure 12: Average % of *S. aloides* turion germination in the full, medium and low light intensity trays between experiment rounds 1 and 2. Light intensity values: full=253µmol, medium=73µmol, low=3µmol (n=60)

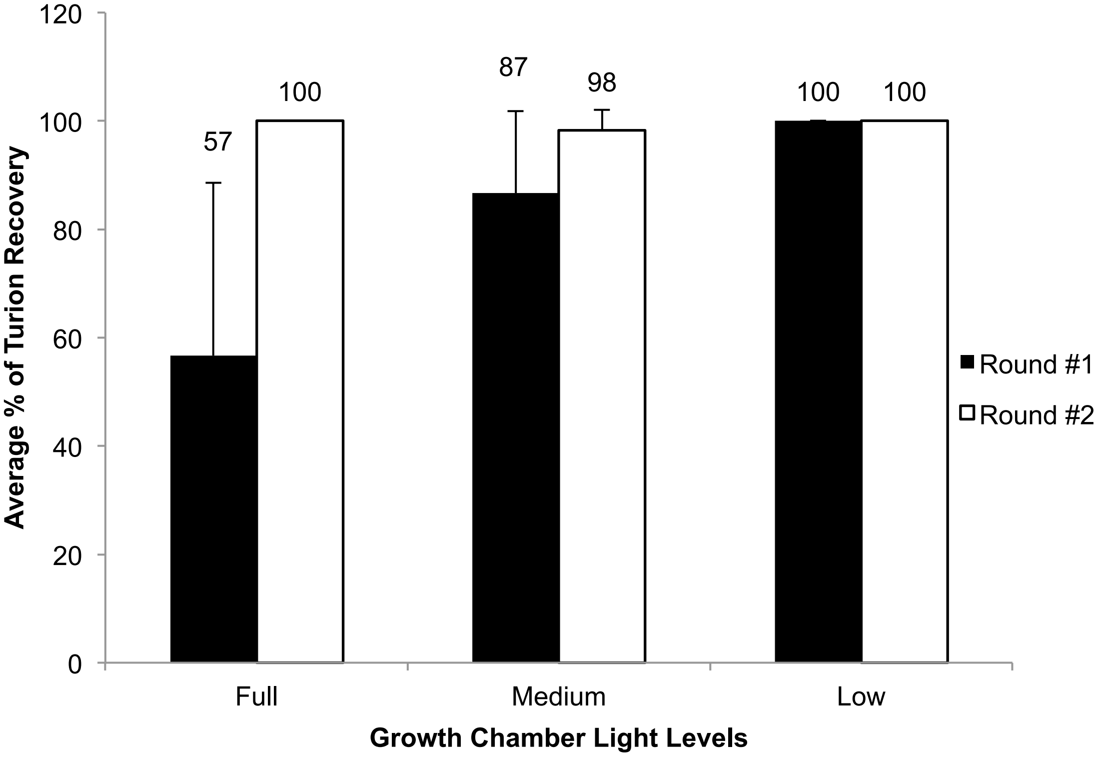


Figure 13: Average % of *S. aloides* turion recovery in the full, medium and low light intensity trays between experiment round 1 and 2. Light intensity values: full= 253µmol, medium=73µmol, low=3µmol (n=60). Average percentage values are presented above each bar

2.4.3 Maximum Depth of Colonization (Z_C)

Results of the linear regressions for the July and August incubation periods can be seen in **Figures 14** and **15**. The coefficients of determination (R^2 values) for the July and August incubations were 0.86 and 0.91, respectively, and showed close fit between the data and the model in both instances. Using the equations of the regression lines (July: y= -0.0008x+ 0.0042 and August: y= -0.007x+0.0043) and inputting a value of 0 for the y variable, the x intercepts were found to be 5.25 and 5.39m. These values represented the depth at which the compensation point was reached for offsets during the incubations. As outlined in McAllister (1991), applying the 20% guideline to the Z_{LC} values allowed for the calculation of Z_C, which was found to be 4.2m in July and 4.3m in August. Applying 95% confidence intervals suggests that Z_C for *S. aloides* could be between 4-6m for both incubation periods. These results are represented in **Figures 14** and **15.** Comparing the Z_{LC} and Z_C predictions for July and August to the light attenuation profile seen in **Table 1**, the approximate light intensity values required for *S. aloides* offsets to reach their compensation points and maximum depths of colonization are: July Z_{LC}=8.02-15.76μmol $m^{-2}s^{-1}$ Z_C=34.68μmol $m^{-2}s^{-1}$ and August Z_{LC}=8.02μmol $m^{-2}s^{-1}$ Z_C=23.02μmol $m^{-2}s^{-1}$. Z_C estimates for July and August suggest that approximately 2-3% of surface light is required for *S. aloides* colonization. A photosynthesis light response curve was generated (**Figure 16**) to compare the relationship between irradiance level and rate of photosynthesis for both incubation dates.

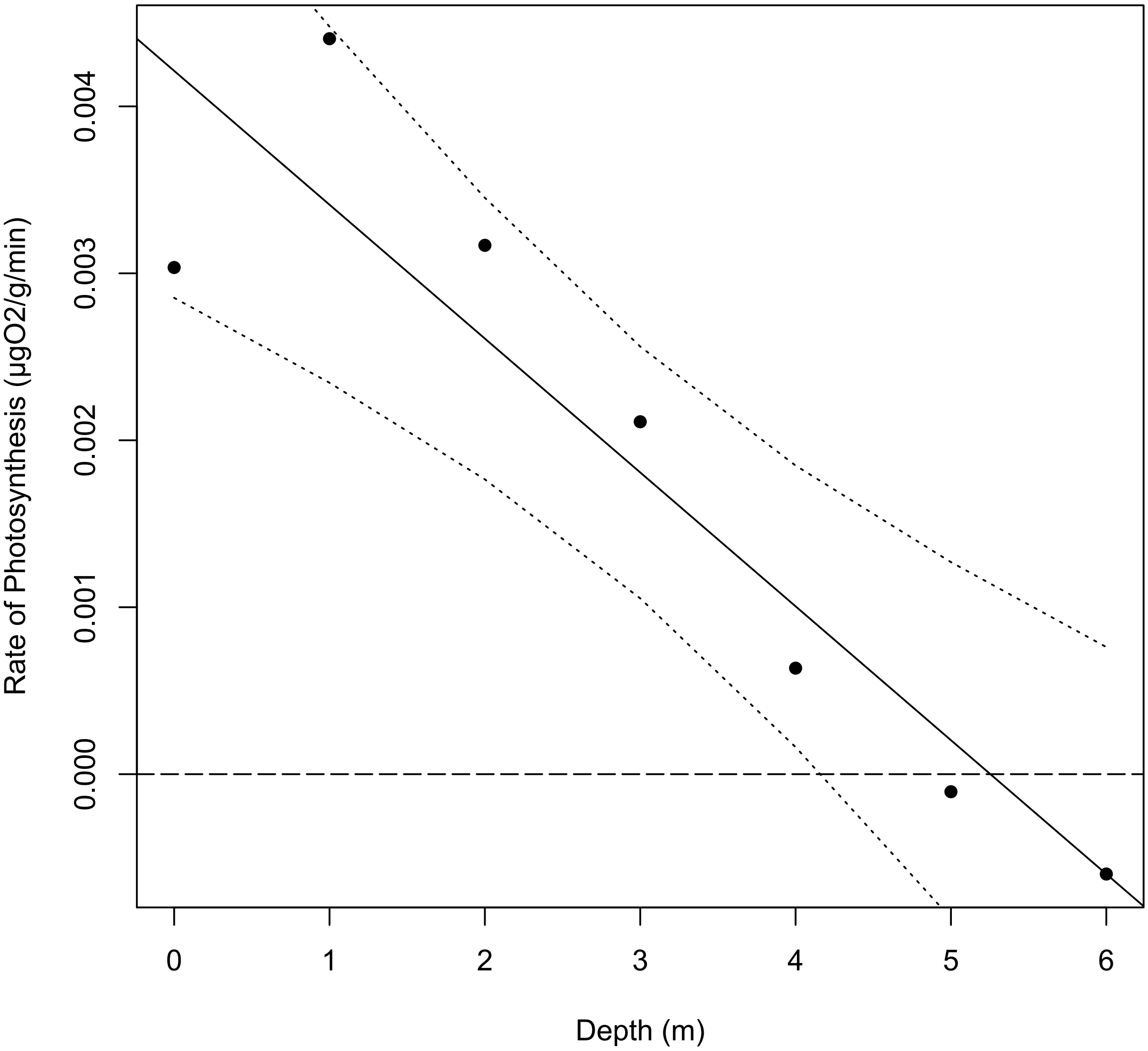


Figure 14: Linear regression of offset rates of photosynthesis from incubation arrays set on July 25th, 2013 (R^2=0.86). The three-hour incubation used changes in dissolved oxygen concentrations to measure net photosynthesis. Where the regression line crosses the dotted line at y=0, the light compensation point is reached (x=5.25m). 95% confidence intervals suggest that the maximum depth of colonization could be between 4-6m

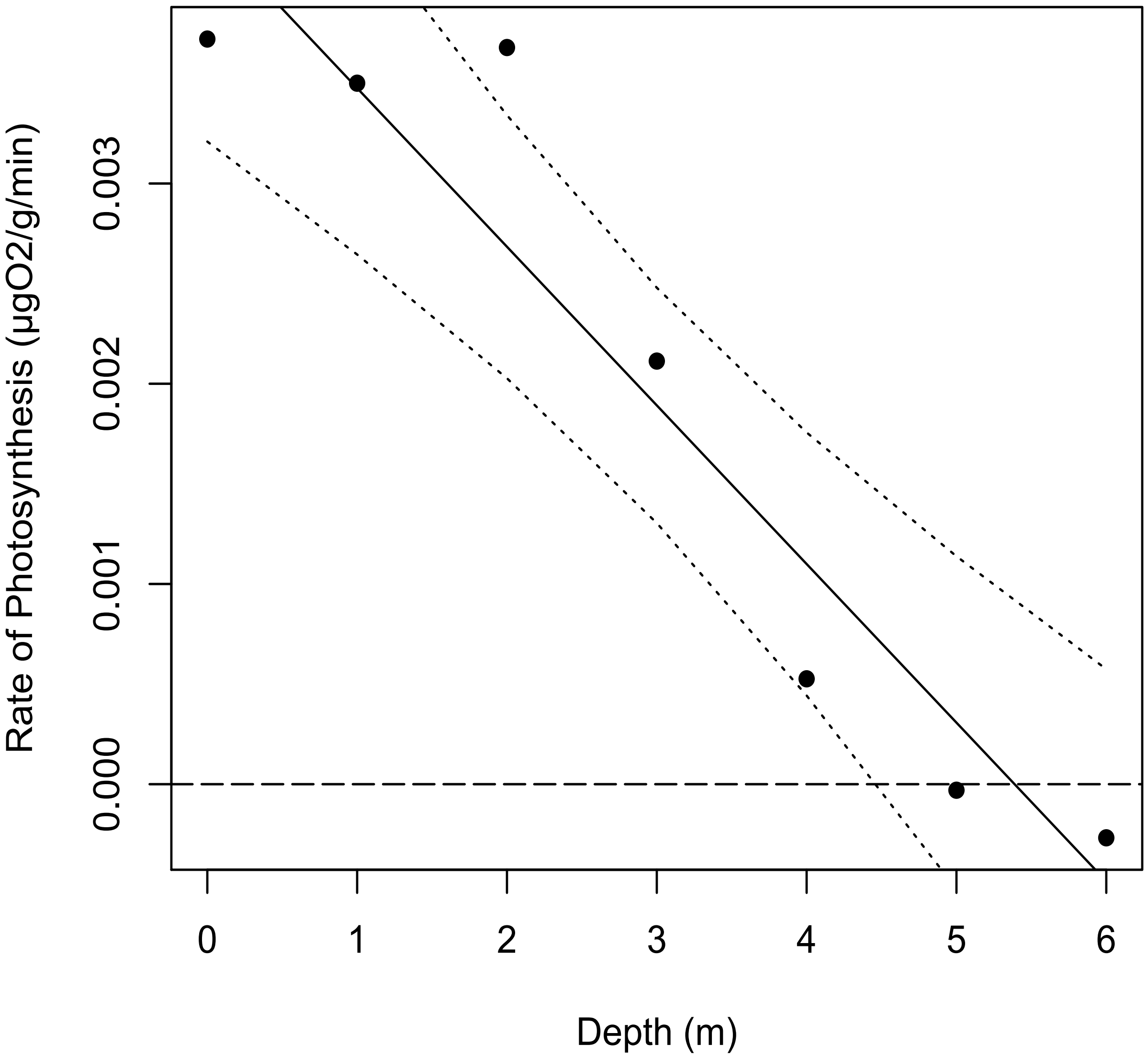


Figure 15: Linear regression of offset rates of photosynthesis from incubation arrays set on August 29th, 2013 (R^2=0.91). The three-hour incubation used changes in dissolved oxygen concentrations to measure net photosynthesis. Where the regression line crosses the dotted line at y=0, the light compensation point is reached (x=5.39m). 95% confidence intervals suggest that the maximum depth of colonization could be between 4-6m

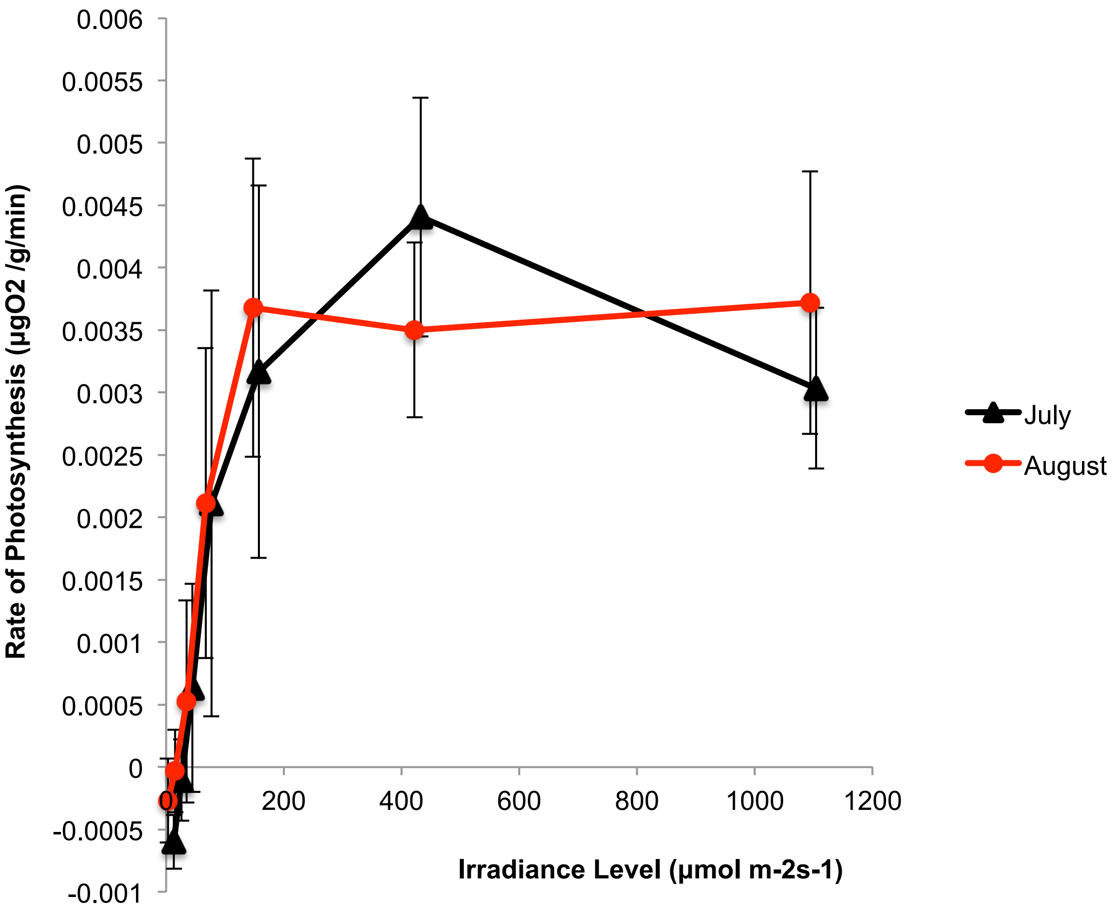


Figure 16: Light response curve for *S. aloides* offsets based upon incubation results from July 25th and August 29th, 2013 and average irradiance levels from measurements taken on: June 18th, July 25th and August 29th, 2013. The curves show that below 200 umol/m^2/sec light intensity, offset response to light is nearly identical in both cases.

2.5 Discussion

At this point, it is unclear what mechanisms have allowed *S. aloides* to spread such distances in the Trent River (>20km since 2008) and whether this species relies more on offsets or turions for long distance dispersion. The results of this chapter indicate that based upon light availability, the ability for either reproductive propagule to survive and begin producing auxiliary colonies can be predicted.

2.5.1 Offset Incubation

The results from the offset incubations in July and August 2013 show that *S. aloides* offsets were capable of photosynthesizing above depths of ~4m, but they showed severe decline in photosynthetic rate below 2m when light intensity drops to around 6% of PAR (**Table 1**). In the lower depth category (2-6m), the offset photosynthetic rates response to depth is, for all purposes, identical between both incubation periods. Below 2m, light attenuation throughout the water column was sufficiently consistent and restrictive to regulate photosynthetic production regardless of sample date.

More diverse interactions between light intensity and photosynthetic rate occurred between the depths of 0-2m in the July and August incubations. As seen in **Figure 9**, rates of O_2 production in July were actually lower at depth=0m than at depth=1m, suggesting that photoinhibition of the offsets occurred under the highest light intensities. In the literature, the effect of photoinhibition on macrophytes has been observed and quantified (Figueroa et al, 1997; Hanelt, 1992; Huppertz et al, 1990; Jimenez et al, 1998), but has also been presented as something that seldom or never occurs and is not overly significant when studying aquatic macrophyte communities (Binzer et al, 2006; Sondergaard, 1988; Witt, 2003).

Photoinhibition is apparent as a reduction in photosynthetic rates observed at light intensities above 1000 μmol $m^{-2}s^{-1}$, but occurs most frequently between 1500-2000 μmol $m^{-2}s^{-1}$ (Hanelt 1992; Huppertz et al, 1990; Titus and Adams, 1979a). The light intensity values required to initiate photoinhibition in the literature were much greater than what was encountered during the July incubations, where the photosynthetic rate decreased after ~450 μmol $m^{-2}s^{-1}$ (**Figure 10**). Full intensity light is generally assumed to be 2000 μmol $m^{-2}s^{-1}$ (Binzer and Sand-Jensen, 2002a; Canfield et al, 1985). This is significantly higher than what was measured in this study, where average surface intensity measurements were 1094.24 μmol $m^{-2}s^{-1}$ ±154.22 SD. Discrepancies in surface light intensity measurements between this study and the assumed value (2000 μmol $m^{-2}s^{-1}$) could be potentially explained since conditions were always partly cloudy on field days when light measurements were taken.

A possible explanation as to why photoinhibition in this study occurred at such low light intensities values could be related to differences in *S. aloides* leaf morphology. Tor Neilson and Borum (2008) studied the differences in *S. aloides* leaf morphology by measuring rates of photosynthesis in leaf fragments taken from emergent plants, submerged plants, immature offsets and germinated turions. They found that emergent leaves had photosynthetic rates three to five times higher than that of any other leaf part. This difference was attributed to the higher specific leaf area and chlorophyll concentration found in emergent leaves as well as their development of thick, waxy cuticles and stomata, which was unique among leaf types (Tor Neilson and Borum, 2008). Immature offsets, as used in this study to measure photosynthesis, are not morphologically adapted to full sun conditions (thin, opaque leaves and reduced chlorophyll concentrations) and as a result, became oversaturated with light. This trend has been reported for submerged-adapted leaf parts of other macrophytes when introduced to high light intensities (Binzer and Sand-Jensen, 2002a; Hussner et al, 2010). Under these circumstances, plants reduce their photon adsorption to prevent damage to their photosynthetic membranes (Lambers et al, 2008), which is what likely occurred to the offsets used in this study.

The results of the August incubation suggest a different light response compared to that observed in July. The plateau type trend seen in the photosynthetic rates of offsets between 0-2m (**Figure 9**) is more reflective of a photosaturation, not photoinhibition relationship (Madsen and Sand-Jensen, 1994). Photosaturation describes the state in which maximum rates of photosynthesis are achieved and maintained by a plant. The light saturation point is the light intensity value required to achieve this state. Photosaturation is evident in light response curves where after reaching peak photosynthetic rates, a plateau effect is generated and these rates can be maintained, but not exceeded, by increasing light intensity values (Herron and Mauzerall, 1970; Mache and Loiseaux, 1973; Madsen and Sand-Jensen, 1994; Sondergaard, 1988, Titus and Adams, 1979a).

Photosaturation has been observed occurring at light intensities ranging from as low as 38 µmol $m^{-2}s^{-1}$ to over 400 µmol $m^{-2}s^{-1}$ (Mache and Loiseaux, 1973; Sondergaard, 1988; van der Bijl et al, 1989). Light saturation points for aquatic plant species also found on the Trent River (*E. canadensis*, *M. spicatum*, *P. amplifolius*, *P. richardsonii* and *V. americana*) range between ~40-180 µmol $m^{-2}s^{-1}$ (Madsen et al, 1991; Madsen and Sand-Jensen, 1994). These values correspond with what was observed during the August offset incubation period in this study where photosaturation was detectable starting at ~157 µmol $m^{-2}s^{-1}$. With all offsets controlled for size and harvest location, it seems likely that the reason why August incubations experienced photosaturation as opposed to photoinhibition, as was seen in the July incubations, relates to seasonal light intensities. Measured light intensities between 0 and 2m were slightly lower in August as compared to July, which could explain why *S. aloides* offsets were able to maximize photosynthesis in August, but were suppressed in July.

Measuring photosynthesis in a closed system (BOD bottles), as was done in this study, introduces other limiting factors when determining rates of O_2 production, such as inorganic carbon concentrations within the test containers. As surface light intensifies, carbon concentration replaces light as the primary limiting growth factor for aquatic plants (White and Reiskind, 1996). DIC availability has been shown to significantly improve aquatic plant growth rates and increase their light saturation point (Madsen and Sand-Jensen, 1994). As available sources of carbon were utilized, rates of photosynthesis may have been adjusted accordingly by the offsets. If this experiment had been carried out in an open environment, carbon limitation would not be a concern. This suggests that the photoinhibition vs. photosaturation responses seen during the July and August experiments were due primarily to light and not another limiting factor such as CO_2.

Future photosynthesis experiments could likely improve the robustness of these results by conducting incubations throughout the entire growing season (ex. from May-September), as opposed to only in the mid to late summer (July and August) as was done in this study. This would be more likely to account for the effect of important photosynthesis dependent factors such as photoperiod, which has been linked specifically to aquatic plant morphology and physiology including growth form development and light compensation point (Pilon and Santamaría, 2002; Salvucci and Bowes, 1981). Pilon and Santamaría (2002) tested the effect of 13, 16 and 22 hour photoperiods on the growth of *Potemogetan pectinatus* in a laboratory setting. They found that plants subjected to the 22 hour photoperiod generated significantly greater biomass than those in the 13 and 16 hour periods. The longer photoperiods also increased stem elongation amongst the plants, but decreased leaf area (Pilon and Santamaría, 2002). Salviccu and Bowes (1981) studied changes in CO_2 compensation points of ten aquatic macrophytes when subjected to 10 and 14 hour photoperiods. In all cases, CO_2 compensation points significantly decreased when changing plant exposure from 10 to 14 hour photoperiods (Salvucci and Bowes, 1981), suggesting that longer photoperiods could increase low light tolerance of aquatic plants.

Day length was decreasing during the offset incubation experiments on the Trent River with a 15:9 photoperiod on July 25th transitioning to 13:11 photoperiod for the second incubations on August 29th. Expanding the incubations to include the full time period between May and September would allow photosynthetic measurement of the plants to occur under photoperiods between 15:9 (May) and 11:13 (September) day-night cycles. Given the relationship described by Salvucci and Bowes (1981) between photoperiod and compensation point, understanding of the photosynthetic capabilities of *S. aloides* plants when exposed to the full range of growing season photoperiods is likely to yield a much more complete picture of this plant's physiological characteristics. Furthermore, conducting future incubations throughout the entire growing season under a full range of photoperiods could help to explain the discrepancies that were found between the actual and predicted maximum depths of colonization for *S. aloides* as described in section 2.5.3.

2.5.2 Turion Germination

Turion germination at all light levels shows that these vegetative structures are capable of germinating at all depths of the Trent River, but would experience higher rates of germination success where light intensities are above 73 μmol $m^{-2}s^{-1}$ or water depth is less than 3m. Although turions germinated at all tested light levels, only propagules in the full and medium intensity exposures matured beyond the opening of bud scales and advanced to rosette and root development (Canning, pers. obs). This is an important consideration for assessing how well turions might be able to establish new *S. aloides* colonies or repopulate areas following disturbances.

Despite the potential for germination success, continued growth and development of turions released into low light conditions (<3 μmol $m^{-2}s^{-1}$) would likely cease. Results of the offset incubation experiment show that light compensation point was reached at ~1% of surface light. Although turions germinated at ~0.3% of surface light in the low light trays, it can be assumed that no active growth could take place after germination at these light levels. This effect has been reported for *S. aloides* seeds within its native range, where germination occurred in dark conditions, but further seedling development required increased light levels and aerobic conditions (Smolders et al, 1995b). The restriction of turion development based on the absence of light was also observed during the present experiments as only turions in the high and medium light trays formed rosettes and roots (Canning, pers. obs).

Turion weight was found not to be a factor in germination success and the similarity in the measured wet weights of the randomly collected turions (range= 0.42g, mean= 0.46g ±0.06g SD, n=180) could suggest that the size of these propagules is naturally controlled or standardized by the plants. Using a ratio developed by Erixon

(1979) for converting wet to dry weight of *S,aloides* plants (dry weight = 6.5% of wet weight), converted dry turion weights from Ontario plants were on average 50-90% larger than average dry weights measured from Eurasian populations (Toma, 2012). Given that the conversion factor developed by Erixon (1979) was related to *S. aloides* rosettes and not turions and that quantifying turions as wet weights as opposed to dry weights introduces some degree of error, these are approximate comparisons only.

The relationship between propagule size and the survival/growth of aquatic and terrestrial plants has been well studied (Spencer et al, 1987). In general, larger propagules from a specific plant species are associated with increased biomass production and viability due to their ability to store more energy reserves in the form of sugars and starch (Ismail et al, 2009; Marshall, 1986; Smolders et al, 1995a; Wes, 1980). Analysis of different types of tissues from *S. aloides* plants has shown that turions contain the highest concentrations of starch of any vegetative material from this species (Efremov and Sviridenko, 2012). The weight similarities among *S. aloides* turions taken from Lake Seymour in this study (range: 0.42g, mean: 0.46g ±0.06, n=180) could indicate an optimal propagule production size based on the maximization of starch concentrations, available resources and environmental conditions.

Turion decomposition was observed in both rounds of the turion exposure experiment and was only evident in trays exposed to full and medium light. Rates of turion recovery were much higher in the second round as compared to the first and could be related to the maturity of turions collected later in the year, although no significant differences in turion size were found. Multiple studies of *S. aloides* populations in Europe describe the “disappearance” of turions from sediment banks, despite observed production in the fall and winter (Kornatowski, 1979,1983; Renman, 1989a). Smolders et al (1996b), studied *S. aloides* turions in a lake in the Netherlands and described finding evidence that turions were being produced, but only very rarely the actual propagules themselves. They hypothesized that the relatively high rates of nitrogen accumulation in the turions made them attractive targets for herbivory from birds and microorganisms, although no specific species were listed (Smolders et al, 1996b). The trays used in the germination experiments in this thesis were not assessed for the presence of potential turion herbivores that could have been responsible for the varying rates of turion recovery. Weissflog and Sager (2016) also noted this occurrence when studying sediment samples taken adjacent to *S. aloides* colonies in Ontario, where no turions from the target species were found despite the presence of turions from other species like *P. crispus*. Although no explanation is given for the disappearance of these propagules, this could indicate that released turions are not able to accumulate in the sediment and form propagule banks for future population regeneration.

A secondary explanation for the disappearance of turions could be related to buoyancy. A study by Sarneel (2013) showed that up to 92% of turions remained floating for over 180 days (~6 months) following their release. In a flowing system like the Trent River, this would provide ample time for dispersal away from parent plants and could have accounted for some of the lack of recovered turions by Weissflog and Sager (2016), who sampled only within/ directly adjacent to *S. aloides* stands. It seems unlikely, however, that 100% of turions would escape the confines of the patch and the sediment sampling. Furthermore, 84% of the floating turions at the end of the study by Sarneel (2013) had developed leaf structures, which indicates that turions are capable of germinating and developing into a rosette while floating, which would prevent them from being classified as a turion if they were collected. Despite this, significant turion buoyancy during the germination experiments in this thesis was not observed and all turions were submerged within 24 hours following placement within the micro-climate chamber.

2.5.3 Light Compensation Point (Z_{LC}) and Maximum Depth of Colonization (Z_C)

The Z_{LC} for a given aquatic plant species can be directly related to its ability to tolerate low light conditions (Washington and Sidnei, 2003). At the compensation point, when the effects of photosynthesis and respiration are balanced, the light intensity reaching the photosynthetic tissues is just enough to maintain the continued survival of the plant, but not enough to allow for carbohydrate storage needed to enable substantial tissue growth or reproductive viability. Aquatic plants exposed to light levels below their compensation points show a progressive decline in biomass in relation to the length of time spent under those conditions (Bowes et al, 1977). In such conditions, plants are forced to utilize their carbohydrate stores just to persist and cannot achieve a positive net carbon balance and accumulate energy reserves for growth (Bonan, 2002; Myers and Kitajima, 2007). Access to sufficient energy reserves plays a significant role in the survival of aquatic plant species exposed to low light and adverse conditions because stored carbohydrates are relied upon heavily to endure high stress periods (Best and Visser, 1987). The storage and use of these energy reserves is also directly related to the ability of aquatic plants to compete in diverse vegetation communities on a long-term basis (Titus and Adams, 1979b). A low light compensation point enables *S. aloides* to photosynthesize under low light conditions and at lower irradiance levels than prominent competing species (**Table 2**). Note that *S. aloides* has a lower light compensation point than most of the other listed species, including that of *H.verticillata*, a highly invasive species from the same family classification. Thus, the ability of *S. aloides* to continually store reserve sugars and starches and produce plant biomass gives it a heightened capacity to colonize consistently low light habitats.

Reported Z_{LC} values for aquatic plants vary greatly depending upon the species. They have been reported to be as low as 0.2 µmol $m^{-2}s^{-1}$, as high as 55 µmol $m^{-2}s^{-1}$ and nearly everything in between (Binzer and Sand-Jensen, 2002a; Madsen and Sand-Jensen, 1994; Necchi, 2004; Olesen et al, 2002; Søndergaard, 1988; Spencer and Ksander, 2001). The light compensation points (Z_{LC}) for *S. aloides* offsets were reached at water depths of 5.25m in July and 5.39m in August, corresponding to light intensity values between 15.76 µmol $m^{-2}s^{-1}$ and 8.02 µmol $m^{-2}s^{-1}$ (0.7-1% of surface PAR). Although the light compensation of *S. aloides* has not been previously studied, the values observed in this study fit within the lower range of measured values described in the literature for other species. **Table 2** provides a summary of Z_{LC} values for comparison between *S. aloides* and other native and invasive plants.

Table 2: Comparison of light compensation values for S. aloides and other native and invasive species. Using the McAllister (1991) Z_C to Z_{LC} ratio rule and light measurements from Table 1, the maximum depth of colonization for these species in the Trent River was also predicted (Z_{LC} values taken from: Madsen and Sand-Jensen, 1994; McAllister, 1991; Sand-Jensen and Madsen, 1991; Spencer and Ksander, 2001; Van et al, 1976; Washington and Sidnei, 2003)

Species (Status native=^{N}, invasive=^{I})	Light Compensation Point Z_{LC} (µmol $m^{-2}s^{-1}$)	Predicted Maximum Depth of Colonization (Z_C) in the Trent River (m)
Stratiotes aloides ^{I}	8.02-15.76	4-6
Ceratophyllum demersum ^{N}	7-35	3-4.5
Elodea Canadensis ^{N}	3-17.8	4-4.5
Vallisineria americana ^{N}	18	3.5-4

Potemogetan pectinatus N	44.7	2.5-3
Potemogetan gramineus N	9.9	4-4.5
Myriophyllum spicatum I	35	3-3.5
Cabomba caroliniana I	55	2-2.5
Hydrilla verticillata I	15	4-4.5

Light compensation values play an important role in determining the maximum depth of colonization (Z_C) for an aquatic plant species. The Z_C of a macrophyte has been shown to be primarily dependent on light availability regardless of plant type and the geographical location of the lake (Canfield et al, 1985). The light compensation point of a plant can decrease or increase the maximum depth at which a species can grow and survive depending upon the light intensity required to maintain a positive rate of photosynthesis. A species with a low light compensation point can continue photosynthesizing at lower irradiance levels; therefore, since light attenuation is strongly related to increasing water depth (Barko and Smart, 1981; Kenworthy and Fonesca, 1996) a plant with a low light compensation point can colonize habitat at depths that are potentially unreachable by shade intolerant species.

The Z_C of a macrophyte species does not necessarily equal the depth at which light compensation is reached. At its light compensation point, a plant is merely existing, not amassing energy stores or reproducing. The maximum depth of colonization reflects the depth at which a plant is not only surviving, but also storing energy and actively producing new individuals to increase the spatial presence and distribution of the genet. This means that in theory, Z_C must always be less than a species' light compensation point to allow for positive rates of photosynthesis to occur.

Although the relationship between Z_C and Z_{LC} is recognized, it has not been quantified (Bodkin et al, 1980) and no concrete rule exists for deriving Z_C from Z_{LC}.

Generally, Z_C estimations are set based upon percentages of surface light required for plants to grow. The Z_C for macrophytes is reported to be between 1 and 4% of PAR (Hudon et al, 2000; Schwartz et al, 2000; Sculthorpe, 1967; Washington and Sidnei, 2003), but it has been shown to range as high as 15-33 % of surface light (Bodkin et al, 1980; Chambers and Kalff, 1985; Sand-Jensen and Sondergaard, 1981). Using this literature, determining the Z_C for *S. aloides* becomes highly variable depending upon which study is used as a reference for the % of PAR required for growth. For example, given the range of light intensity values required for macrophytes to grow that are reported in the literature, lower depth limits for *S. aloides* colonization could fall anywhere between 1 and 33% of surface light. This results in a Z_C estimate between 1 and 5m, which is too wide a range to provide enough accuracy for pinpointing priority monitoring and management areas on the Trent River. Using percentages of PAR to predict Z_C has been shown to be highly subjective based upon the plant species, lake morphology and the particular study in which the estimations were carried out. Specific light meter and photosynthesis measurements for each species are required to provide accurate maximum depth of colonization predictions (Caffrey et al, 2007).

As outlined in the Methods section, the calculation of Z_C from Z_{LC} in this study was made based upon findings from McAllister (1991) where the distribution of *V. americana* in Green Bay, Lake Michigan was found to be approximately 20% less than its measured light compensation value (McAllister, 1991). Applied to the present study, this method yields a Z_C value of 4.2-4.3m, although this is much too specific to be applied across the entire system. Instead, conservative 95% confidence intervals suggest that the Z_C for *S. aloides* offsets is between 4-6m. Based upon light availability alone, *S.*

aloides is capable of colonizing over 90% of the total surface area of the Trent River. Referring to **Table 2,** Z_C values for *S. aloides* are generally lower than other plant species that are present within the Trent River. This indicates that *S. aloides* has a greater propensity to establish under low light conditions and can continue photosynthesizing at water depths lower than the majority of other competing species. This is a huge advantage in terms of increasing overall distribution and reducing competing plant community diversity, especially at greater depths.

Spencer and Ksander (2001) studied the relationship between light compensation and competition between *Potemotegan pectinatus* and *Potemogetan gramineus*. In their study, *P.gramineus* was found to cause significant displacement of *P.pectinatus* when introduced into macrophyte beds. By measuring the light compensation point of each plant, they found that *P.gramineus* was able to continue photosynthesizing at lower light intensities due to its lower Z_{LC} (9.9 μmol $m^{-2}s^{-1}$) value compared to that of *P.pectinatus* (44.7 μmol $m^{-2}s^{-1}$). The ability of *P.gramineus* to continue photosynthesizing beyond that of *P.pectinatus* resulted in an 83% reduction in *P.pectinatus* occurrence after only two months of *P.gramineus* exposure (Spencer and Ksander, 2001). In relation to the Trent River, if lower compensation values can be directly related to the displacement of other species, this could explain how *S. aloides* has been able to colonize such expansive areas of the system and reduce native biomass so significantly. Furthermore, this suggests that taking a “do-nothing” approach to *S. aloides* management in hopes that other species will eventually out-compete and displace it, will yield unsatisfactory results.

Based upon the results of the *S. aloides*-light intensity study by Harpenslager et al (2015), some inferences can be made about the Ontario population in the Trent River. In their study, they were able to determine that the maximum growth and colonization potential for *S. aloides* could only be reached at light intensities at or above 130 µmol $m^{-2}s^{-1}$. Beneath this, *S. aloides* plants were found not to be able to form floating canopies (which reduces their ability to outcompete local vegetation) and only exhibited slow water body colonization tendencies (Harpenslager et al, 2015). Comparing this threshold value to those measured on the Trent River (**Table 1**), indicates that light intensities equal to or greater than 130 µmol $m^{-2}s^{-1}$ were only reached in water depths between 0-2.5m. Plants found in water deeper than 2.5m can be hypothesized to have increased chances of remaining as submerged rosettes. Applying the findings made by Harperslager et al (2015) to the Trent River suggests that plants found growing within the 0-2.5m depth class pose the greatest risk for becoming emergent, outcompeting local plant communities, population expansion and propagule creation and should be considered management priorities. Unfortunately, the vast majority of the Trent River falls within the 2.5m depth limit, meaning that most of the *S. aloides* populations could be classified as having a high invasive potential. Furthermore, considering the results of Harpenslager et al (2015), the distinction between emergent, highly aggressive and submerged, non-colonizing populations cannot be made based solely upon water depth and light intensity. *S. aloides* stands in the Trent River are comprised of mixed emergent and submerged plants with no clear separation between to two and frequently, the water column position of *S. aloides* plants appears to change on a daily basis. Although light availability plays a critical role in *S. aloides* growth, there appears to be additional compounding factors responsible for fully determining its water column positioning and colonization ability.

When applying the predicted Z_C values to actual conditions found on the Trent River, considerations need to be made regarding where photosynthetic rates for the *S. aloides* offsets were measured and the differences between community and phytoelement photosynthesis. To assess photosynthetic rates of offsets at a full range of depth values, incubation arrays were set in deeper, open water areas of the Trent River. The measured rates were indicative of a plant that had been released or displaced from a larger colony and could be floating independently away from its genet throughout the water body. In this case, turbidity and zoo/phytoplankton communities likely represented the most significant sources of light attenuation throughout the water column. When offsets are initially released, however, they are likely to encounter significantly greater rates of light attenuation due to shading from *S. aloides* leaves within the confines of the colony, than what would have been encountered in the open water incubation arrays. This suggests that if the incubations took place within dense plant beds, photosynthetic rates would be lower and the measured Z_{LC} and predicted Z_C values would likely be higher than what was recorded in this study, based upon the impact of vertical shading within *S. aloides* stands.

Indeed, most studies on photosynthetic rates utilize individual phytoelements such as leaf parts or single plants, like the offsets used in this study (Binzer and Sand-Jensen, 2002a; Madsen and Sand-Jensen, 1994; Spence and Chrystal, 1970; Spencer and Ksander 2001). Measuring the productivity of phytoelements, however, has been shown to give false impressions of photosynthesis when applied on a community scale (Binzer and Sand-Jensen, 2002b; Binzer et al, 2006; Pedersen et al, 2013). Binzer et al (2006) reviewed 190 photosynthesis experiments and found both productivity and light compensation point values were greater for single phytoelements when compared to whole communities. Average photosynthetic rates were 8.8 µmolO_2/m^{-2}/s^{-1} for phytoelements and 14.2 µmolO_2/m^{-2}/s^{-1} for communities and light compensation values were 21.7 µmol $m^{-2}s^{-1}$ and 119 µmol $m^{-2}s^{-1}$ (Binzer et al, 2006). Binzer and Sand-Jensen (2002a) measured photosynthesis of the macroalgae *Fucus* serratus (toothed wrack) found similar results. Light compensation points measured in communities were higher compared to that measured for thallus pieces, 22 µmol $m^{-2}s^{-1}$ to between 67 and 175 µmol $m^{-2}s^{-1}$, respectively (Binzer and Sand-Jensen, 2002a). The differences between phytoelement and community photosynthesis reflect the facts that leaf fragment productivity is not proportional to whole-plant photosynthetic rates (Koyama and Kikuzawa, 2009) and that shading occurs within dense macrophyte beds (Binzer et al, 2006; Binzer and Sand-Jensen, 2002b). Plants growing on or near the surface of the water can capitalize on high light availability to precipitate greater rates of photosynthesis, but individuals growing closer to the bottom are subject to increased rates of light attenuation through leaf shading and show lower rates of photosynthesis. At the community or colony level, plants subject to shading and lower productivity balance the high rates of photosynthesis seen in individuals closer to the surface. When measured as a whole, productivity is averaged throughout (Binzer et al, 2006; Binzer and Sand-Jensen, 2002b; Pedersen et al, 2013). As the number of individuals increases, so does net productivity until eventually, the density of plants is so great that vertical shading begins to cause a decline in maximum photosynthesis (Binzer and Sand-Jensen, 2002b). In this manner, the density of a *S. aloides* stand begins to work against itself and could indicate that this species is self-limiting at dense concentrations.

Using submerged offsets provides an estimate of Z_C for *S. aloides* in the Trent River. Since offsets are a major dispersal agent for this species, this provides some understanding as to their ability to independently colonize new areas of the Trent River. The selection of plant parts not optimized for high light intensity photosynthesis is not necessarily a limitation when determining the maximum depth of colonization. The fact that the predicted values are probably greater than the actual depth limits allows ecosystem managers to invoke the precautionary principal when selecting monitoring techniques and survey areas to ensure the highest degree of confidence in detecting and controlling a species.

Employing a light based model to generate depth distribution predictions for *S. aloides* suggested a high degree of colonization suitability in the Trent River (~90%). Actual populations on the Trent River, however, did not appear to fully reflect this figure. Monitoring surveys conducted by the Ontario Ministry of Natural Resources and Forestry (OMNRF) and the Ontario Federation of Anglers and Hunters (OFAH) indicated that *S. aloides* was never detected in the Trent River below ~3.5-4m in depth, as opposed to the 4-6m limits estimated through the offset incubations. This demonstrates that although light availability plays a key role in the depth limitation of this species, there must be other compounding factors in effect. By referring to the most recent distribution map for this species in the Trent River (**Figure 5**), *S. aloides* populations can be consistently seen to follow and be limited by the contours and boundaries of the actual TSW channel. *S. aloides* plants have been found growing adjacent to, but never fully within this waterway feature. It can be hypothesized that this is related to several factors: depth, bottom morphology and current forces within the channel.

In general, the region of the Trent River where *S. aloides* is currently present can be divided into two depth classes: within the actual TSW channel, which ranges anywhere from 3.5-12m in depth and all the surrounding areas where depth ranges in a much more gradual 0-3.5m. Given the estimations of the incubation experiment (4-6m depth limit), much of the TSW channel is too simply deep to support growth by *S. aloides* propagules or rosettes. Furthermore, historical dredging from the creation of the TSW means that the bottom morphology of the Trent River shifts from deep, organic sediment in the shallower areas, to limestone flats, course gravel and rock deposits within the actual shipping channel itself. It is recognized in the literature that *S. aloides* prefers soft sediments that can accommodate its weak rooting structure (Cook and Urmi-Konig, 1983; Tor Nielson and Borum, 2008) and high nutrient demand (Cook and Urmi-Konig, 1983; Kufel et al, 2010). The bottom structure within the TSW channel is highly inhospitable to plant growth in general, let alone the species in question. Strong current following the course of this channel also likely prevents plants from being able to establish in the hard bottom type and could probably help to flush propagules/plants from this area into slack water bays adjacent to the channel.

Further study could indicate that perhaps some of the factors that are responsible for the population control/decline of *S. aloides* within its native range might also serve to pose restrictions on its distribution within Ontario. Throughout Europe, *S. aloides* has declined and even disappeared from much of its native range (Roelofs, 1991; Smolders et al, 1996a, 2003). Explanations for this decline have been attributed to: internal eutrophication, iron deficiency and sulphide and ammonium toxicity (Roelofs, 1991;

Smolders and Roelofs, 1996; Smolders et al, 1996a, 2003) related to the rise of intensive agriculture and industrial land-use practices (Smolders et al, 2003).

Eutrophication causing increased competition from free-floating plant species such as *Lemna sp.* (duckweed) and filamentous algae is seen as one factor for inhibiting *S. aloides*. These species can form light suppressing canopies before *S. aloides* rosettes have a chance to ascend to the surface in the spring (Smolders et al, 2003). Despite the documented ability of this species to suppress autotroph growth through nutrient uptake (Kufel et al, 2010) and allelopathic compound production (Cook and Urmi-Konig, 1983; Mulderij et al, 2005, 2009), severe eutrophication must be able to encourage competing free-floating macrophyte and algal growth beyond what the competitive traits of this species can handle. In the Trent River, both *Lemna trisulca* (star duckweed) and algal populations can be observed growing within *S. aloides* stands, but clearly not in the densities needed to initiate *S. aloides* decline.

Iron deficiency in the sediment, combined with increased concentrations of sulphide and ammonium has been shown to be toxic to *S. aloides* plants (Roelofs, 1991, Smolders et al, 2003). Changes in water/sediment ion composition have been attributed to the diversion of historic hydrological pathways within the past 50-100 years in which highly alkaline water with high sulphite concentrations is introduced into natural systems (Smolders et al, 1996a, 2003). Iron deficiency promotes the decay of *S. aloides* roots, while sulphide and ammonium exposure reduces the overall vitality of *S. aloides* plants leading to increased susceptibility to plant herbivory, pathogens and eventually causing death (Smolders et al, 1996a, 1996b, 2003). Roelofs (1991) showed that exposing *S.*

aloides plants to sulphide and ammonium resulted in increased plant decay over the winter and was lethal to this species (Roelofs, 1991).

Without doing a specific analysis of the water and sediment in the Trent River, it is difficult to determine in what concentrations iron, sulphide and ammonium are present within the TSW where *S. aloides* is located and what their role is in determining the distribution of this species. Future studies for quantifying to what extent conditions in the Trent River resemble those in Europe where this species is in decline would likely provide insight for ecosystem managers as to the health of this population and their potential for continued colonization within Ontario.

CHAPTER 3: Experimental management of *S. aloides*

3.1 Introduction

Determining the best approach for managing or removing an invasive plant species can require a wide range of specialized knowledge pertaining to species biology, stage of invasion, appropriate control methods, and impacts to non-target organisms. The complexity of the management strategy can be compounded if the species in question is a recent invader for which no precedent for successful management exists. In these situations, it is necessary to prepare for rapid management action before the species can become established (Netherland and Schardt, 2014).

Generally, eradication is much more successful when control actions are applied as soon as possible after discovering the presence of an unwanted invasive species. This type of rapid response to a new invasion may be feasible if there is sufficient, relevant information available about the target species to inform management actions. Attempts to remove or control long-standing, widely distributed invasive plant species are more likely to succeed if detailed research on biology, dispersal mechanisms and potential susceptibilities is used to improve and adapt current control methods (Myers et al, 2000; Simberloff, 2003).

Clear procedures are essential for minimizing the negative impacts of an invasive species regardless of the stage of colonization. After identifying that there is a need for anthropogenic intervention to reduce or remove a species, an integrated management plan needs to be developed (Simberloff et al, 2005). An integrated management plan identifies the:

- overarching goal with respect to the invasive plant species (e.g., eradication, containment, threshold maintenance, alleviation of problematic outcomes, monitoring, or inaction) (Locke et al, 2010)
- key management goals (e.g., control actions, monitoring and prevention, education) (Lock et al, 2010; Myers et al, 2000; Simberloff et al, 2005)
- specific, measurable objectives used to achieve each goal (Myers et al, 2000)
- plan for monitoring and follow up

Management goals related to control of an invasive species also need to be based on consideration of factors such as available funding, time constraints and location(s) of the target species (Simberloff et al, 2005). It is typically necessary to tailor management plans to specific areas of infestation (Simberloff, 2003). The specific objectives identified to achieve management goals related to control constitute the action plan and serve as indicators that are used to determine if the related goal has been achieved (Hobbs, 2007; Madsen, 2014; Myers et al, 2000). Selection of appropriate control methods depends upon the management goals, target species biology and local site conditions (Myers et al, 2000; Simberloff, 2003). Combining more than one treatment method into an integrated management plan can provide a holistic approach to invasive plant control that can be adapted for a wide range of circumstances and environmental considerations.

Accounts of early aquatic plant management in the U.S. date back to the 1800's. Efforts to control *E. crassipes* populations in the southern states are considered the foundation for modern techniques on aquatic invasive species management across the globe (IFAS, 2016). At present, a variety of aquatic plant management techniques suited to all types of species and situations have been identified. These methods can be grouped into three main categories: biological, physical, and chemical. Each of these methods is described below.

Traditional biological control methods involve the introduction of natural enemies, organisms such as arthropods, nematodes and/or pathogens, from the target species' native range into the non-native environment (Cuda, 2009). The success of biological control depends on identification of an appropriate biocontrol agent that can effectively control the target species without negatively impacting non-target species. At the present time, there is no known biological control organism for *S. aloides*.

Physical control methods typically require the least amount of specialized knowledge to administer, while still providing effective plant control (Bellaud, 2014). These methods include: hand-pulling, mechanical harvesting, suction dredging, benthic barriers and water level drawdown and may be suited for applications ranging from single plant removal to whole lake restorations (Bellaud, 2014; Haller, 2014). The effectiveness of these techniques is primarily operator dependent and less sensitive to environmental factors than chemical or biological controls, where factors such as water temperature, turbidity and climatic suitability can impact treatments. Manual removal is an especially good option for involving the public and utilizing volunteer efforts. Most physical control methods require plants to be moved away from the site and disposed of on shore, resulting in immediate visual reductions in target species biomass (Bellaud, 2014; Haller, 2014).

Chemical control methods involve the application of one or more herbicides directly onto a target species, which then kills the plants in a variety of ways such as defoliation, enzyme inhibition and/or photosystem disruption (Netherland, 2014). Aquatic herbicides work through either contact (localized) or systemic activities (Netherland, 2014). Contact herbicides are absorbed by a plant and only impact the area to which they were directly applied (Netherland, 2014). Systemic herbicides translocate throughout the plant moving from the leaves to the roots, stems, tubers, and so on, via the plant's xylem and phloem (Netherland, 2014). Aquatic herbicides can be applied using various methods, ranging from personalized backpack sprayers to large boom mounted airboats, amphibious vehicles, and watercraft (Netherland, 2014).

Before being used, herbicides must be reviewed, deemed safe and registered within each jurisdiction where the chemical application is to take place (Health Canada, 2009). There are currently 14 herbicides registered for use on submerged aquatic plants in the U.S. (Netherland, 2014), but only one is approved for use in Ontario (OMAFRA, 2016). Strict regulations determine who can apply herbicides (specialized training and permitting are required), when the chemicals can be applied, and at what concentrations (Netherland, 2014; OMAFRA, 2016).

Herbicides are seen as a fast and cost effective method for aquatic plant control. Compared to physical control techniques, herbicides require fewer resources (i.e., no large harvester assemblies, dive boats, or dredge barges are needed) and can be used to treat large areas within much shorter periods of time (OMNRF, 2013). That being said, most aquatic herbicides are non-selective and will negatively impact both invasive and desirable native plants (Netherland 2014a; OMAFRA, 2016). They may also disrupt fish and macroinvertebrate communities and create the need for temporary water use restrictions for shoreline residents (Netherland, 2014). The proper selection and application of herbicides to minimize impact to non-target organisms is essential for responsible aquatic plant management (Netherland, 2014).

Given the regulations surrounding aquatic herbicides in Ontario, the selection of a suitable herbicide for this research required little consideration. The only herbicide registered for aquatic use in Ontario is Diquat (trade name Reward®). Diquat (6,7-dihydrodipyrido [1,2-α:2',1'-*c*} pyrazinediium dibromide) is categorized as a bipyridylium herbicide. Diquat is a contact herbicide that works by inhibiting photosystem 1 and causing defoliation amongst treated individuals (Alberta Agriculture and Forestry, 2016; Glomski et al, 2005; Netherland, 2014). Light availability is therefore an indirect factor in determining Diquat effectiveness (Hess, 2000). It is a broad-spectrum herbicide (non-selective) and will kill a range of aquatic plants including most species found in the Trent River. This product was shown to be effective for *S. aloides* control in laboratory trials (Netherland, 2013), but no quantitative evidence exists regarding its field effectiveness against this species, especially in moving waters like the Trent-Severn Waterway.

Due to its strong affinity for the clay mineral anions found in soil, Diquat is considered a low risk for groundwater contamination (Abu Ghalwa et al, 2012). Diquat released by decaying aquatic plant membranes binds with negatively charged particles in the soil (Simsiman and Chesters, 1976). Diquat bound to clay minerals or other organic materials in the soil is subject to slow microbial degradation (Netherland, 2014;

Simsiman and Chester, 1976) and the potential for Diquat bioaccumulation is low (Siemering and Hayworth, 2008).

Although not metabolized in fish, animals or humans, the presence of Diquat (through contact with skin, inhalation and/or ingestion) has been associated with increased risk of cataracts in humans and laboratory animals (Abu Ghalwa et al, 2012; Clark and Hurst, 1970; Simsiman and Chesters, 1976). Some invertebrates commonly found in the littoral zone of freshwater lakes are sensitive to Diquat (e.g., *Hyalella azteca*-amphipod and *Asellus communis*-isopod) although levels of susceptibility vary greatly (Williams et al, 1984). Using a probability-based model, Campbell et al (2000) concluded that under all conditions tested, Diquat posed very low risk to organisms because the speed with which Diquat is dissipated in aquatic environments ensures that virtually none of the herbicide remains available for uptake by organisms. Chung et al (2008) reported that of seven herbicides tested for toxicity to *Palaemonetes pugio* (Grass shrimp) larvae, Diquat was the least toxic.

Since *S. aloides* had never been encountered or managed in Ontario, little information existed regarding the effectiveness of control procedures. To test the impacts of multiple control options, both physical and chemical methods were selected (no effective biocontrol agents are known for *S. aloides*) and treatments were applied at different times throughout the year. The two types of plant control tested in this study were manual raking and herbicide (Diquat) application and the application timings were spring and fall.

Manual/hand raking was chosen as the physical control technique best suited for *S. aloides* removal in this study. With large floating rosettes and weak root structures anchored in soft sediment, raking was anticipated to be sufficient for harvesting plants from the treatment areas. The small size of study plots selected in this study (0.25 ha) and abundance of stumps and underwater obstacles prevented the use of large mechanical harvesters. The selectivity of manual raking was hypothesized to reduce non-target plant impact and promote greater local community recovery as opposed to more invasive mechanical methods.

Herbicide application was selected for its ability to provide even coverage of the treatment plots and a potentially higher degree of control than that of manual raking. The capacity to efficiently treat large areas using Diquat could mean that if successful, this treatment method could easily be scaled to treat most of the Trent River. The broad-spectrum selectivity of Diquat was considered a detriment to desirable plant species and substantial impacts to local vegetation communities were expected, but this negative impact was considered a necessary risk for providing a widespread treatment option.

The impact of application timing on the effectiveness of physical and chemical control measures was also assessed in this study by administering treatments in the spring and the fall as opposed to the summer months. The main reason for the temporal separation of treatment times was to take advantage of seasonal factors that could enhance treatment effectiveness and reduce impacts on non-target species, as described below.

In the spring, few local vegetation species have started to produce leafy biomass that can interfere with herbicide uptake by the target species, reduce visibility for manual raking and increase non-target species impacts. Although flow rates are higher at this time of the year, Sand-Jensen and Pederson (1999) found that water velocities in dense plants beds (like those found in the Trent River) are greatly reduced compared to open water areas, which may help to mitigate this factor and improve herbicide exposure time. Treatment of *S. aloides* plants in the spring could also reduce the number of individuals able to produce turions later in the year, potentially helping to delay colony regeneration within those sites. Netherland et al (2000) showed that treatment of another turion producing species (*P. crispus*) in the spring resulted in an 86% decrease in turion density in the fall as compared to a 40% reduction from summer spraying.

In the fall, the biomass of most local vegetation has declined within the water column, as these plants exist mainly as seeds, tubers, turions etc., buried in the sediment. Flow rates at this time of year in the Trent River are generally lower than those encountered in the spring, which could aid in increasing Diquat-*S. aloides* contact time. These conditions could potentially increase treatment efficacy by increasing water clarity and reducing the potential of non-target species impacts from raking and chemical applications.

The objectives of this chapter are to: i) select, test and assess the effectiveness of two treatment regimes for the eradication of *S. aloides*, ii) monitor the impacts of the treatments on local plants and assess short-term community recovery and ii) describe the impact of *S. aloides* colonization in the Trent River in terms of macrophyte community dynamics and the formation of new species assemblages.

3.2 Methods

3.2.1 Site Selection

To test the effect of the treatments on *S. aloides,* four 2500m^2 (0.25ha) study plots were designated within areas of the most abundant *S. aloides* populations (as close to 100% *S. aloides* coverage as possible) and a fifth 2500m^2 (0.25ha) plot was set up as a reference site. Sites for the treatment plots were selected based upon the need for high and even percent cover by *S. aloides,* with enough distance between plots to eliminate the chance of interference between treatment methods. As a precaution against herbicide drift, treatment sites were kept at a minimum of 250m apart and chemical application areas were always located downriver of hand-pulling plots.

The direction of *S. aloides* range expansion has appeared to follow the predominate water flow/ current direction in the Trent River (which flows west to east throughout Ontario), so the reference site was established as far as possible upstream (west) of the initial *S. aloides* introduction site, while still maintaining comparable vegetation types. A limitation of this portion of the Trent River is that west of the *S. aloides* populations, the TSW becomes relatively narrow and channelized as it enters Rice Lake and the water body morphology and plant communities different quite significantly as a result, making consistent site selection challenging. Compounding this issue is that there are TSW locks immediately both west and east of the *S. aloides* infestation, limiting available sampling area. Essentially, the reference site was selected to be as far away as possible from any known *S. aloides* populations, while still maintaining comparable plant communities and river morphology (depth, fetch, current etc.), as well as being within the same lock section for ease of accessibility and boat access. The final location for the reference site was established 2km upstream (west) of the closest *S. aloides* location. The locations of the treatment and sites on the Trent River are shown in **Figure 17**. To ensure equal percent cover of *S. aloides*, as well as to make sure that similar local plant communities were present within and across the treatment and reference sites, site selection was informed by previous Trent River macrophyte sampling (Canning, 2012), performing visual surveys of potential sampling sites through snorkeling and utilizing an underwater camera.

The plots were delineated using handheld GPS units and floating buoys to mark the perimeter of the areas. **Table 3** summarizes the treatment variables and the control regime for each study plot. The effects of the treatments were compared based on the reduction in *S. aloides* biomass and the impact on native species biomass, diversity and community dynamics. The reference site was used to provide baseline data for macrophyte communities on the Trent River that had not been exposed to colonization by *S. aloides.* It served as a way to compare changes in local plant community biomass and diversity to the *S. aloides* treatment sites, as well as to help understand the impact of non-treatment effects to plant communities on the Trent River (ie. seasonal biomass and community composition shifts). In this study, local plant communities were considered to represent all plants both exotic and indigenous, other than *S.aloide.*

Table 3: Test plot treatment types, application timing, site code and treatment dates.

Plot	Treatment	Site Code	Application Timing
1	Physical	SPhys	Spring: June 4-9, 2013
2	Chemical	SChem	Spring: June 11, 2013
3	Physical	FPhys	Fall: October 2-6, 2012
4	Chemical	FChem	Fall: October 26, 2012
Reference (5)	None		

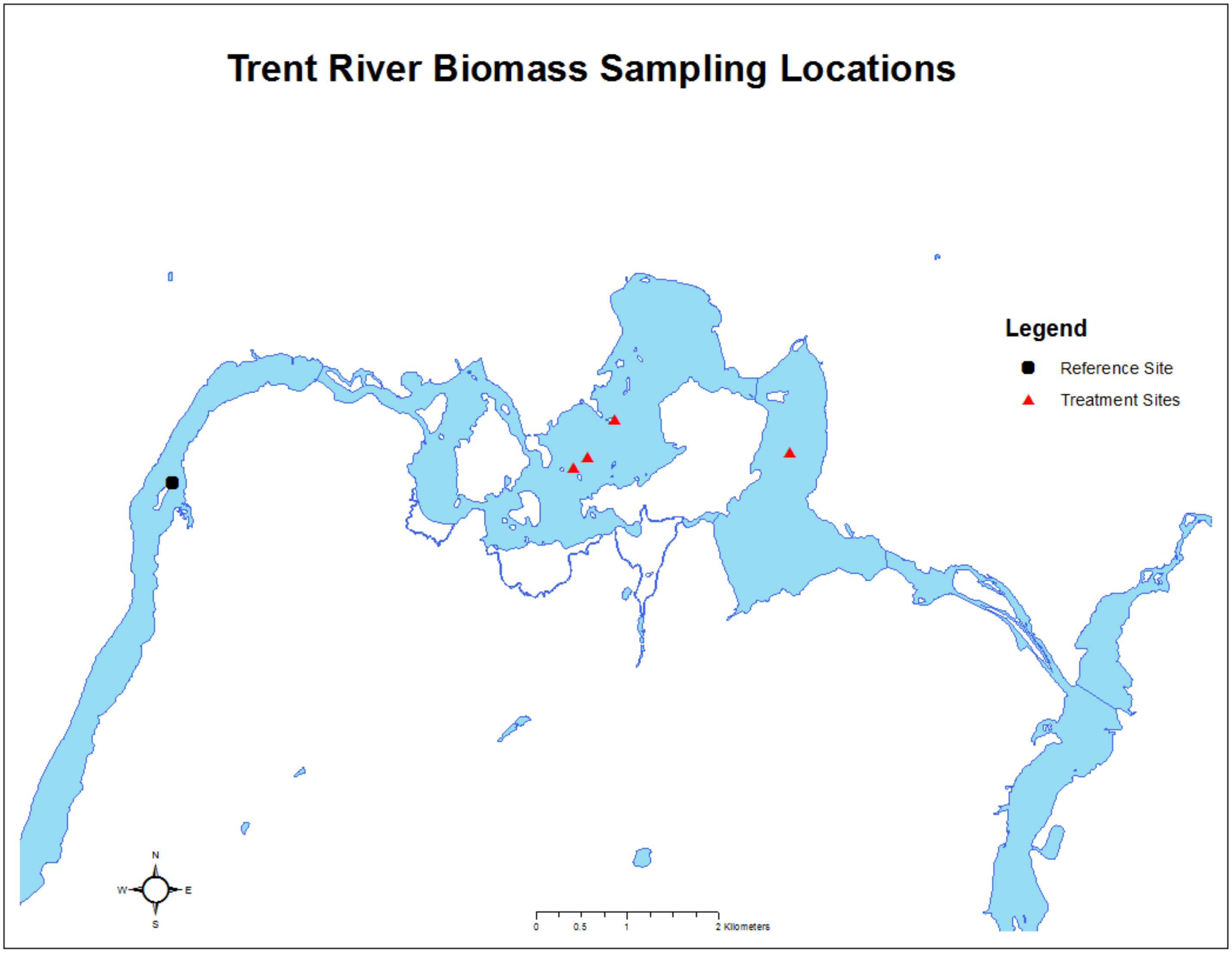


Figure 17: Map of Trent River/ Lake Seymour sampling locations. The reference site was placed upstream of any known *S. aloides* colonies, above the point of introduction for this species

3.2.2 Goals for *S. aloides* Management

At the onset of this study in 2012 *S. aloides* had been present in the Trent River for about four years and could be considered at an early-mid stage of invasion. There was no prior history of colonization in North America and at the time, *S. aloides* distribution in Ontario was limited to a system that was controlled through the large number of waterway locks. Management goals outlined in the Ontario Ministry of Natural Resources and Forestry (OMNRF) Integrated Management Plan (IMP) for *S. aloides* in Ontario (2013) were adopted and used to determine effectiveness of the control treatments used in this study. In the provincial IMP, the primary management goal was complete eradication (defined as >99% reduction in biomass) of *S. aloides* in the Trent River, with secondary objectives set at a minimum of 75% biomass reduction (OMNRF, 2013).

3.2.3 Physical Control

Physical control of *S. aloides* was accomplished through manual raking using long-handled, large toothed rakes (**Figure 18**). A boat was anchored within the treatment plot and *S. aloides* plants were harvested (and brought aboard the boat) until all plants within the plot were collected or until the water clarity declined sufficiently so that it was impossible to determine the location of additional target plants. In the latter case, the water was allowed to settle while the boat moved to another location and then returned at a later time to continue removal. The harvested plants were stored in the boat while on the water and were transferred to compostable bags and properly disposed of after transfer to shore (**Figure 19**). This process was repeated until all of the *S. aloides* plants within the designated treatment plots were removed.

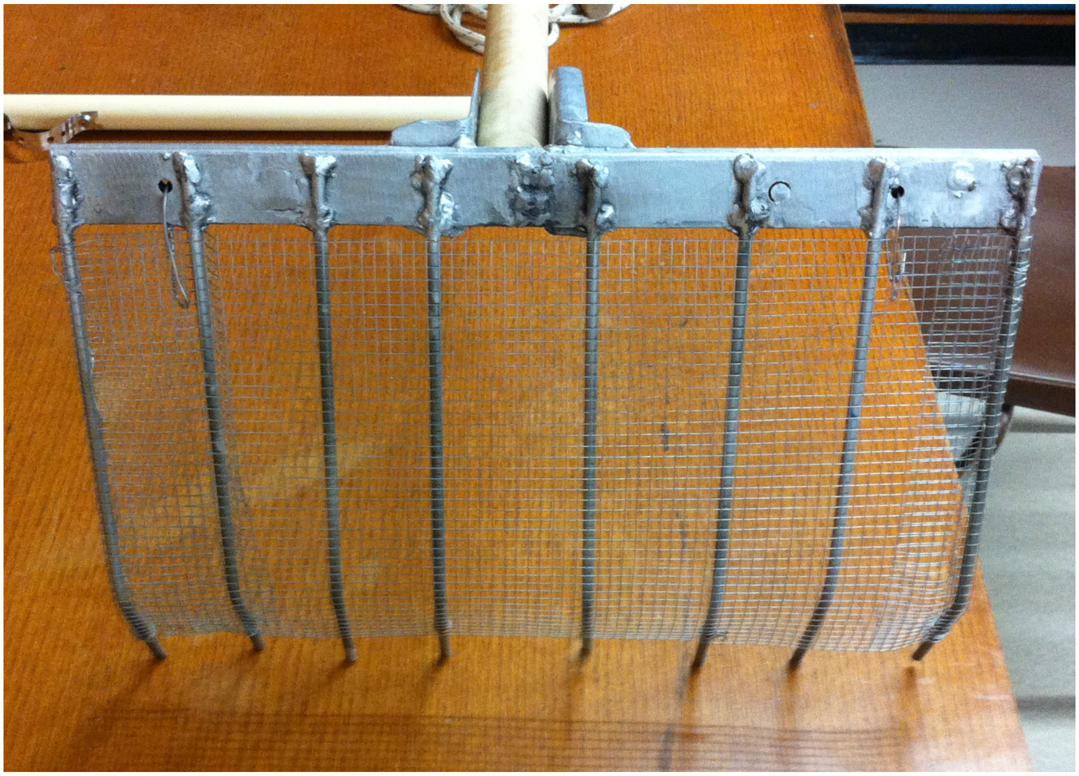


Figure 18: Custom built, long tooth rake used for manual control of *S. aloides* on the Trent River

Figure 19: Collection of *S. aloides* plants from fall 2012 hand pulling. Plants were collected within the boat while on the water and transferred to disposable bags and composted once on shore

3.2.4 Chemical Control

Licensed aquatic herbicide application professionals applied Diquat in the trial chemical plots. Using an amphibious vehicle, the individuals applying the herbicide were instructed to follow GPS tracks within the marked trial plots to ensure full coverage of each plot. Diquat was to be applied in liquid form at concentrations of 18.3 L/ha at water depths ≤1.5m and 25-29.3 L/ha in areas more than 1.5m deep. A two-foot wand extending below the surface of the water was used to better target submerged *S. aloides* plants and maximize plant tissue contact by the herbicide. Equipment used for the Diquat application is shown in **Figure 11**.

Figure 20: John Graham Agro-Services/ Port Britain Chemical performing fall 2012 Diquat application. Using an amphibious vehicle (Argo 6x6) they are able to traverse the debris filled waters of the Trent River. The chemical was applied with a long, extendable wand to maximize plant-chemical contact

3.2.5 Plant Sampling

In each of the four 2500m^2 (0.25ha) treatment and one reference sites, six 50m transects were set up at 10m intervals, orientated northwest to southeast. Every 10m along each transect (starting at the 0m mark), a 0.25m^2 quadrat was placed on the sediment and all above ground plant material within was removed and placed in an individual bag corresponding to that particular site and transect location. This procedure was carried out a total of four times: once before the treatment regimes were applied (pre-treatment) and once every two months following the treatments (post-treatment) until the end of the growing season (June, August and October respectively). Using this method, 36 individual plant samples were collected per site, per sampling period. In total, 720 plant samples were collected over the course of the experiment.

When sampling plots that included *S. aloides*, their tendency to grow in dense, multi-layered mounds required that an additional step be taken to ensure collection of plants only within the quadrat. A small wire loop was attached to the side of the standard $0.25m^2$ quadrat, just large enough so that a ¾" CPVC pipe could be threaded through it. The 3m CPVC pipe was inserted through the loop and then secured into the sediment allowing the quadrat to be held securely in place, while permitting movement up and down on the pole. When encountering *S. aloides*, the quadrat could be placed on top of the plants as they were collected and then pushed down to the next layer of vegetation, all the while maintaining quadrat position and sample accuracy. The depth of each quadrat was recorded.

Plant samples were placed in coolers and taken back to the lab. Plants were then sorted by species for richness (diversity) quantification and the total wet weight for each species was measured. Biomass was recorded in wet as opposed to dry weights due to the shear size of *S. aloides* plants and the volume of each plant sample. Oftentimes, individual plant samples would weigh in excess of two kilograms. Multiplying this sample weight by the 36 samples that were collected per site meant that it was not feasible and well beyond the capacity of standard ovens to dry all the plant material while maintaining sample freshness and integrity. All latent moisture, roots, epiphytes, sediment etc. were removed before weighing. If the samples were not processed immediately, they were refrigerated and processed within 24 hours. All reported and analyzed plant biomass values reflect wet weights. A list of all the plant species identified during plant sampling can be found in **Table 4**.

Table 4: List of species encountered during aquatic plant sampling in the Trent River, with growth form and plant status

Species	Growth Type	Status
Elodea canadensis	Submerged	Native
Vallisneria americana	Submerged	Native
Ceratophyllum demersum	Submerged, free-floating	Native
Nymphaea odorata	Floating leaf	Native
Nuphar variegata	Floating leaf	Native
Najas flexilis	Submerged	Native
Ranunculus longirostris	Submerged	Native
Zosterella dubia	Submerged	Native
Megalodonta beckii	Submerged	Native
Utricularia vulgaris	Free-floating	Native
Chara sp.	Macroalgae	Native
Lemna trisulca	Free-floating	Native
Potamogetan robbinsii	Submerged	Native
Potamogetan richardsonii	Submerged	Native
Potamogetan zosteriformis	Submerged	Native
Potamogetan pusillus	Submerged	Native
Potamogetan amplifolius	Submerged	Native
Potamogetan crispus	Submerged	Exotic invasive
Potamogetan praelongus	Submerged	Native
Myriophyllum spicatum	Submerged	Exotic invasive
Stratiotes aloides	Submerged/Floating	Exotic invasive

3.2.6 Sample Analysis

Plants weights for samples collected in the treatment sites were compiled and divided for into two categories: 1) *S. aloides* and 2) the local community (defined as all other species present regardless of their native or exotic status in Ontario), for each sample period. Plant samples from the reference site were compiled into a single local community category. All compiled plant category weights were converted into g/m^2 for analysis. Species richness and SWI values were compared as average richness/SWI per quadrat. Species richness was measured by counting the total number of plant species identified in each plant biomass sample. Shannon-Wiener Index values were generated for each biomass sample and were calculated using:

$$SWI = -\Sigma[(P_i)\times(\ln(P_i)]$$

where,

Σ= sum
P_i= proportion of total individuals belonging to the ith species

Species associations were quantified as frequencies of occurrences by determining the number of times a species was present within a quadrat sample where *S. aloides* was also present, across the site. In the reference site, frequency of occurrence for the local plant species was measured by counting the number of times a species was recorded within a biomass sample, throughout the entire site.

A limitation of this experiment was that there were few continuous patches of *S. aloides* of the consistent size and required percent cover at the time this study was conducted (refer to section 3.2.1 for more details on site selection). This meant that each treatment could only be replicated once. In an attempt to account for this, each of the six transects within a respective site were treated as a pseudo-replicate and biomass within a site was calculated as g/m^2/transect. Between and within treatment biomass and diversity measurements (species richness and Shannon-Wiener Index values) were compared using a one-way ANOVA with an appropriate post-hoc test when significant results were reported. Where necessary, data were transformed to meet statistical test assumptions and/or non-parametric comparisons were made using the Kruskal-Wallace test. All data analysis and figure generation was conducted using Microsoft Excel and R version 2.15.1. A summary of which statistical methods were used for each comparison can be found in Appendix 3.

3.3 Results

3.3.1 Treatment Effects on *S. aloides* Biomass

There were no significant differences in the pre-treatment *S. aloides* biomass (ANOVA; $p>0.05$) between the four treatment plots, showing that all four sites were of equal *S. aloides* densities at the onset of the study. Changes to *S. aloides* biomass from all sample periods can be seen in **Figure 21**.

Following the spring and fall treatment applications, no significant reductions (ANOVA; $p>0.05$) in *S. aloides* biomass were observed at any sites at the first post-treatment sampling period in June 2013 (**Figure 21**). This suggests that either each treatment had an approximately equal effect on *S. aloides* biomass or that there were consistent site/environmental factors present that dictated the degree of *S. aloides* decline apart from treatment type.

Significant *S. aloides* biomass increases from June 2013 levels were observed during the second post-treatment sample period in August. Increases were encountered in the spring physical (Tukey post hoc; p=0.02), spring chemical (Tukey post hoc; p=0.0004) and fall physical sites (Tukey post hoc; p=0.05), whereas no significant biomass regeneration occurred at the fall chemical site (Tukey post hoc; p>0.05). This suggests that this treatment was sufficient to delay immediate *S. aloides* re-colonization of the site. The biomass of *S. aloides* at this time was at or above pre-treatment levels, including in the fall chemical plot and was consistent across every site. During the August 2013 *S. aloides* sampling, there was a high incidence of partially decomposed rosettes sampled in the spring chemical site, which may be evidence that the Diquat treatment did not fully kill each plant and allowed regeneration to occur from small offsets. Only the bases of the rosettes were present, but there were oftentimes small, healthy offsets growing from the core of the rotten plant (**Figure 26**).

During the third and final post-treatment sampling period in October 2013, *S. aloides* biomass showed some significant differences from pre-treatment levels (ANOVA; p=0.024), where biomass rose to well above its September 2012 level in the spring chemical site (Tukey post hoc; p=0.00004). *S. aloides* regrowth in this site (spring chemical) was significantly greater than in the spring physical (Tukey post hoc; p=0.01) and fall physical plots (Tukey post hoc; p=0.001), but was equal to that of the fall chemical treatment.

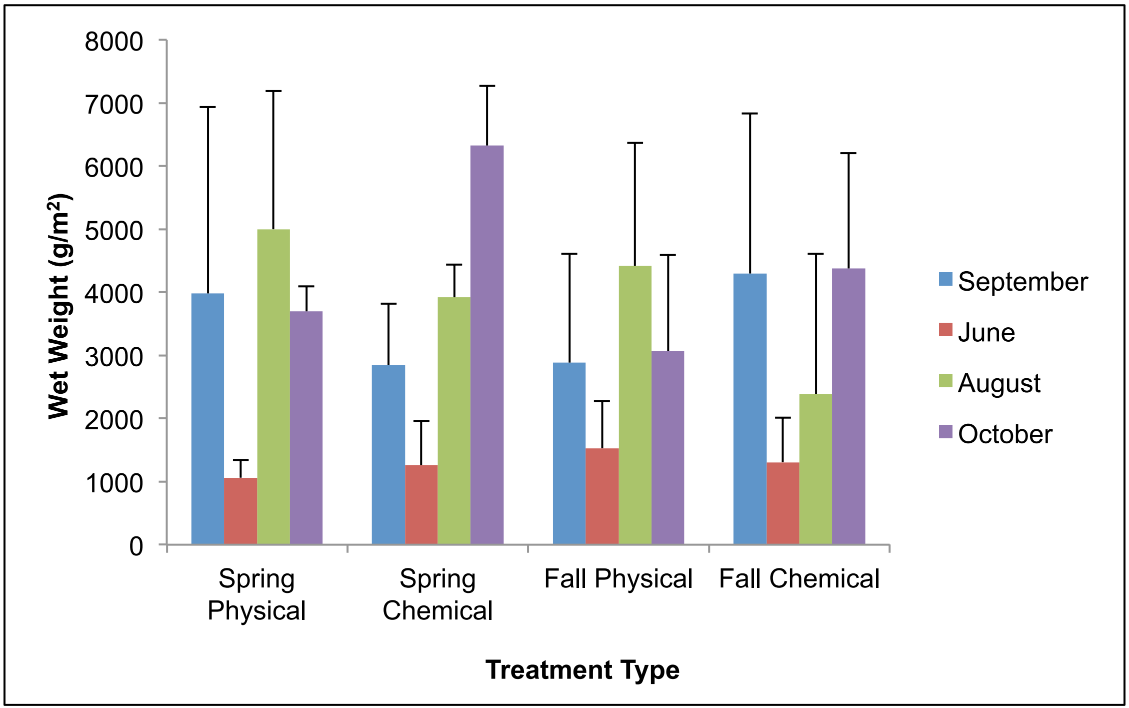


Figure 21: Comparison of average *S. aloides* biomass values (g/m^2, n=36) between the different treatment types (spring physical, spring chemical, fall physical and fall chemical) during the four sample periods. Spring treatments were applied in June 2013 and biomass sampling occurred in September (pre-treatment), June (1 month after treatment), August (3 months after treatment) and October (5 months after treatment). Fall treatments were applied in October 2012 and biomass sampling occurred in September (pre-treatment), June (8 months after treatment), August (10 months after treatment) and October (12 months after treatment).

Figure 22: June 2013 spring chemical plot one day after Diquat application. Note the brown hue already visible on the emergent leaves signifying tissue damage. Submerged individuals do not show the same level of impact as emergent plants

3.3.2 Treatment Effects on Local Community Biomass

Pre-treatment sampling of the local plant community showed that the biomass of plants in the reference and treatment plots at that point in time were not significantly different (ANOVA; $p>0.05$). However, local plant biomass was greater in the spring chemical site than the fall physical (Tukey post hoc; $p=0.01$) and fall chemical (Tukey post hoc; p=0.02) sites. Changes to local plant biomass between sample periods can be seen in **Figure 23**.

Local community biomass in the first post-treatment sample period generally showed no changes from pre-treatment and reference plot levels, except in the fall chemical site, which was found to have significantly greater biomass levels compared to both the reference plot (Tukey post hoc; p=0.01) and the spring physical (Tukey post hoc; p=0.02) treatment plot at that point in time. Changes to local plant biomass in the fall chemical site during this sample period were driven by abundance increases from *P.zosteriformus* and *C. demersum*. By the second post-treatment monitoring period, no significant differences (ANOVA; p>0.05) in total local plant biomass were detected between the treatment and reference plots.

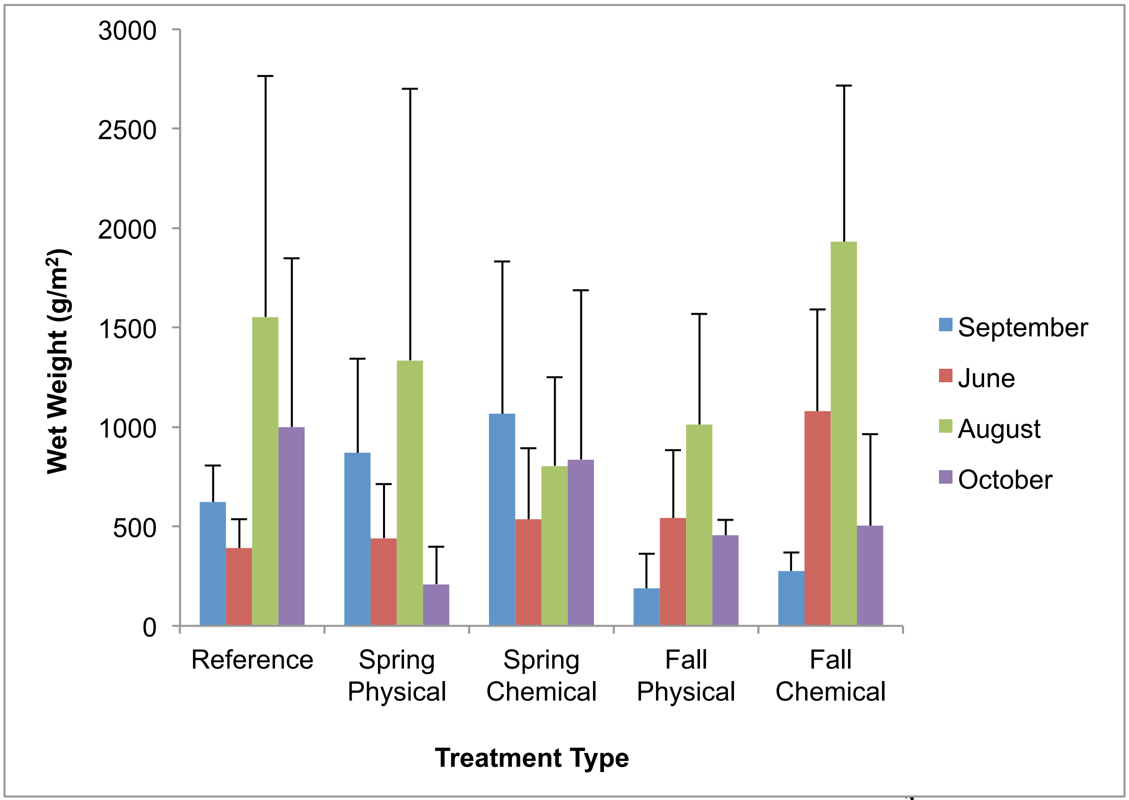


Figure 23: Comparison of average local plant biomass values (g/m^2, n=36) between the different treatment types (spring physical, spring chemical, fall physical and fall chemical) and reference site during the four sample periods. Spring treatments were

applied in June 2013 and biomass sampling occurred in September 2012 (pre-treatment), June 2013 (1 month after treatment), August (3 months after treatment) and October (5 months after treatment). Fall treatments were applied in October 2012 and biomass sampling occurred in September (pre-treatment), June (8 months after treatment), August (10 months after treatment) and October (12 months after treatment)

No significant differences between local plant biomass in the treatment and reference sites were observed in the final sample period (ANOVA; p>0.05). Trends from within site comparisons in the reference/treatment plots from August-October predominantly show declines in local biomass. Significant decreases in local plant biomass occurred between the August-October sample periods in the spring physical (Tukey post hoc; p=0.005) and fall chemical sites (Tukey post hoc; p=0.001). October biomass returned to pre-treatment levels (Tukey post hoc; p>0.05) in the spring chemical, fall physical and fall chemical sites, but was found to be significantly less than what was collected in September 2012 in the spring physical site (Tukey post hoc; p=0.007). This decrease in local plant abundance coincides with and is likely the result of the significant *S. aloides* biomass increase observed in that site (spring chemical) during that sample period (**Figure 21**).

3.3.3 Treatment Effects on Community Diversity: Species Richness and Shannon-Wiener Index (SWI)

Analyses of the impacts of the different control measures on the macrophyte community diversity within the sample plots indicate that few significant effects to species richness (**Figure 24**) and SWI (**Figure 25**) values from the treatments were recorded. Species richness between the treatment and reference sites was found to be equal (Kruskal-Wallis; p>0.05) prior to the application of *S. aloides* treatments in the September 2012 pre-treatment sample period. Following the herbicide and raking applications, species richness remained consistent (Kruskal-Wallis; p>0.05) in the treatment sites when compared to the reference plot during the June and August sampling periods. By the final sampling period in October, there were significant changes in species richness between the treatment sites (Kruskal-Wallis; p=0.001) and richness at that time was found to be higher in the fall chemical treatment site than in the spring chemical site (Tukey Nemenyi post hoc; p=0.001). The maximum species richness value was measured at 9 species/quadrat, which was recorded in samples from the reference plot and in the spring physical treatment site.

Shannon-Wiener Index (SWI) results (**Figure 25**) were similar to that of species richness. SWI values were not significantly different between the treatments sites and the reference plot when sampled in September 2012 before the application of the plant treatments or after the first post-treatment sampling period in June 2013 (Kruskal-Wallis; p>0.05). Diversity index values in the treatment sites remained consistent with those in the reference plot (Kruskal-Wallis; p>0.05) between the June and August 2013 sampling periods. SWI values were significantly different between the treatment sites in August (Kruskal-Wallis; p=0.009) and SWI measurements were found to be greater in the fall chemical (Tukey Nemenyi post hoc; p=0.01) and fall physical (Tukey Nemenyi post hoc; p=0.01) sites than in the spring chemical site. SWI values amongst the treatment sites and reference plot were found to be the same (Kruskal-Wallis; p>0.05) at the final post-treatment sampling period in October 2013. Between-treatment comparisons showed significant differences in October SWI values (Kruskal-Wallis; p=0.001) with greater index values in the fall chemical site compared to the spring chemical site (Tukey Nemenyi post hoc; p=0.003).

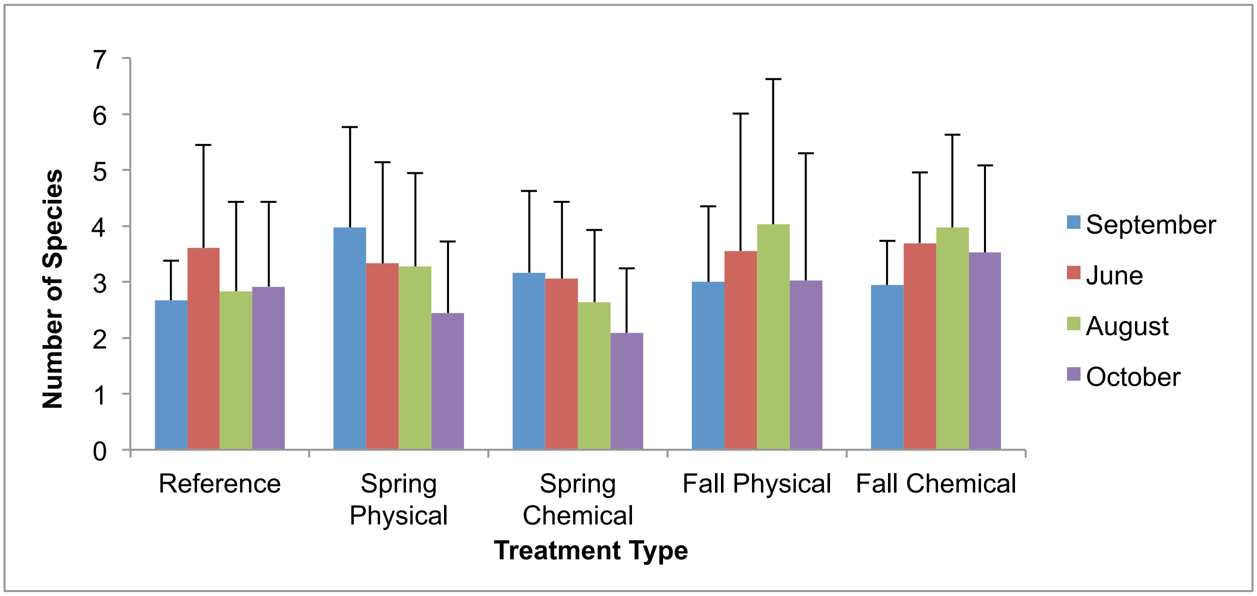


Figure 24: Comparison of average quadrat species richness values between the four treatment sites and reference site (n=36) across the four sampling periods (September, June, August and October)

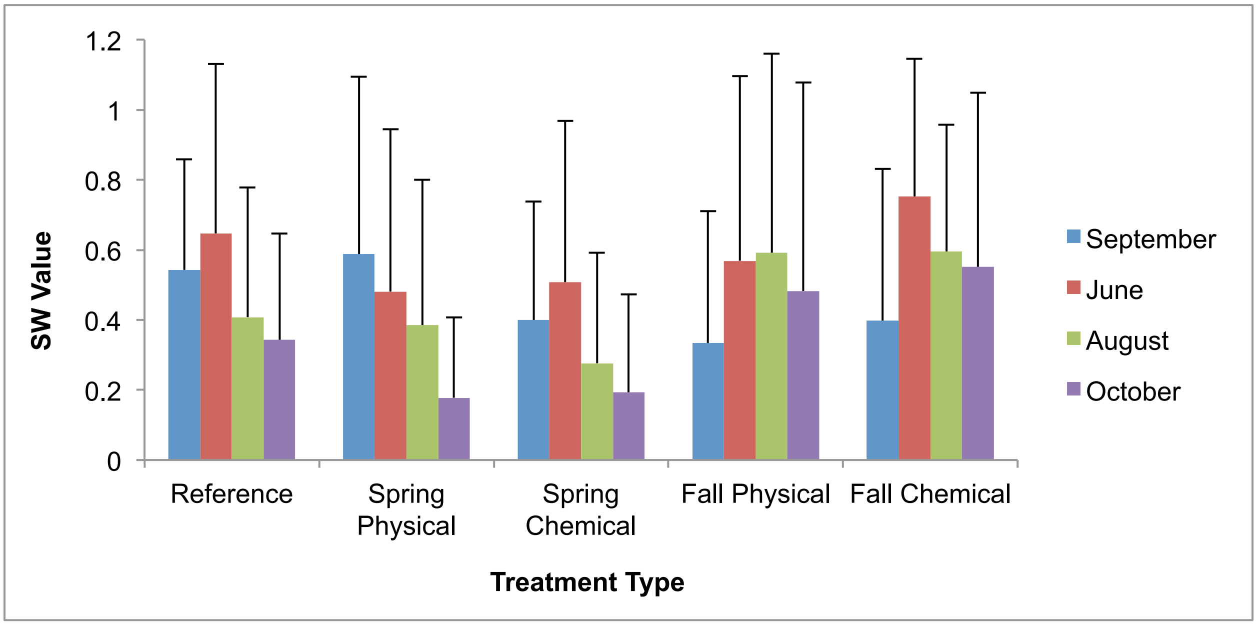


Figure 25: Comparison of average quadrat Shannon-Wiener Index values between the four treatment sites and the reference site (n=36) across the four sampling periods (September, June, August and October)

3.3.4 Community Dynamics: *S. aloides* Species Associations

A measure of the local plant community's response to the invasion of *S. aloides* was determined by identifying the plant species that were most commonly found in samples from sites containing *S. aloides*. **Table 5** lists the seven most frequently occurring species with *S. aloides* in order from highest to lowest frequency along with each species' occurrence in the reference plot for comparison.

Table 5 indicates that community dynamics shifted in the presence of *S. aloides* within the area studied. The most frequently recorded species in the treatment sites was *C. demersum*, which was found in 60% of the samples (on average), as compared to 42% occurrence in reference plot samples. There were no significant changes in *C. demersum* frequency between the pre and post treatment sample periods (ANOVA; p>0.05) in the treatment sites. In the reference plot, *C. demersum* was only the third most commonly occurring species due to the increased presence of the native species *V. americana* and *E. canadensis* in June and the invasive species *M. spicatum* and *P. crispus* in the fall.

Table 5: Species Most Frequently Occurring in Sites with *S. aloides* Compared to the Reference Plot.

Species Key		
Species	**Average Frequency of Occurrence in Treatment Sites**	**Average Frequency of Occurrence in Reference Site**
C. demersum	60%	42%
P.zosteriformus	30%	22%
M. spicatum	24%	56%
P. richardsonii	22%	6%
E. canadensis	20%	38%
V. americana	19%	51%
P. crispus	11%	42%

3.4 Discussion

3.4.1 *S. aloides* Response to Treatment

Effective control of *S. aloides* was not achieved in any treatment plot when considered over time. Short-term reductions in *S. aloides* biomass observed throughout the sample periods could not be fully attributed to the treatment applications alone. Generally, changes in biomass and diversity from the treatments sites were consistent with those in the untreated reference site, suggesting that the treatments did not achieve their desired results and that other factors (ie. seasonal variations) are influencing aquatic plant communities in the Trent River. The only positive impacts towards reducing *S. aloides* populations were seen in the fall chemical site, where no significant increases in *S. aloides* biomass were seen between June 2013 and August 2013, whereas all other sites more than doubled in target species biomass in that same time period. Population regeneration from individuals remaining after treatments and recolonization of treated sites throughout the growing season resulted in abundance levels equal to or higher than pre-treatment values by the final post-treatment fall sample period.

Targets for successful eradication of *S. aloides* have been set at >99% reductions in biomass, with secondary control targets at 75% reductions (OMNRF, 2013). Using the June 2013 post treatment plant samples, where the lowest quantities of *S. aloides* biomass were collected of any sample period, average reductions in biomass from samples collected during the pre-treatment September sampling were: 73% (spring physical), 56% (spring chemical), 47% (fall physical) and 69% (fall chemical) respectively. Of these average initial reductions no treatment type was able to achieve even the minimum control target (75% reductions) as set by the OMNRF. Furthermore, as time after the treatments increased, so did *S. aloides* biomass. Post-treatment sampling showed that left alone or under treatment conditions that do not provide sufficient plant biomass reductions, *S. aloides* was able to double its standing biomass on the Trent River within a year. *S. aloides* biomass measurements at the final post treatment sample periods were equal to or greater than pre-treatment values. This confirms the conclusion that the treatment measures were not effective as even short-term control measures and allowed for extensive recolonization of those sites. Failure to effectively reduce *S. aloides* populations demonstrates the impact of unmeasured variables such as: local environmental factors, lateral recolonization and operator expertise as potent inhibitors of manual raking and/or chemical control efficacy.

One of the aims of this experiment was to test the impact of different treatment application times on *S. aloides.* This meant that there was a difference in the period of time from when a treatment was applied and when the first post-treatment sampling took place between the fall and spring applications. The fall applications, (completed October 2012), were administered ~8 months prior to the spring applications in June 2013. Apart from this initial variation in sample dates, all the sites were sampled using the same post-treatment assessment schedule (June, August and October 2013 monitoring dates). Given these temporal differences, both *S. aloides* and local vegetation within the fall treatment sites would have had those additional 8 months of exposure and recovery time compared to the spring treatment sites, which were sampled within only weeks of the herbicide and manual raking applications. The extant to which this difference between spring and fall treatment-sampling dates influenced how the macrophyte community was able to respond/recover from the treatments is unknown, but should be considered when making conclusions about how the *S. aloides* populations reacted to plant management.

The time/recovery advantage gained by plants in the fall over the spring treatment sites could have also been partially mitigated by the fact that the additional 9 month period was mostly during the winter, when plant growth has drastically slowed or stopped due to non-optimal growing conditions, ice cover etc. The variation in time periods did not appear to severely influence *S. aloides*, as no significant differences in biomass were found between the fall and spring sites in the September 2012 pre-treatment and June 2013 post-treatment sample periods. There were, however, high numbers of partially decomposed rosettes, with healthy offsets attached (**Figure 26**) found in samples taken from the spring chemical site, which were not found in the fall chemical site. Presumably, this could indicate only partial lethality of *S. aloides* rosettes by Diquat and that if the basal node was not completely destroyed, the plants were still able to reproduce. Furthermore, the decomposed rosettes were not found in the fall chemical site which suggests that during the longer recovery period in that site (8 months), the partially killed rosettes either fully decomposed or recovered before they were sampled in June 2013. Since the time period between the treatment and sampling was so much shorter in the spring chemical site compared to the fall chemical sites, evidence of these partially killed rosettes still existed.

Despite *S. aloides* biomass reductions between 47-73% after the first post-treatment sample period, *S. aloides* biomass in all sites was able to return to or exceed pre-treatment levels by the end of the final post-treatment sample period, regardless of treatment type or application timing. The chemical and physical treatment methods used in this study required that unique sets of application procedures and techniques be employed to for each control type in order to provide the desired results. The similar response of *S. aloides* to the different treatments suggests that there were additional factors helping to dictate *S. aloides* recovery and the effectiveness of the treatment methods. In this case, something that was consistent across every treatment site was that the sample plots were established within larger *S. aloides* colonies and were surrounded by *S. aloides* plants. Given the reproductive proficiency of this species, it seems reasonable to conclude that the rapid recovery of *S. aloides* within the treatment sites was driven primarily by recolonization from the adjacent *S. aloides* populations that were in close proximity to the treatment areas. Recording and comparing *S. aloides* biomass based upon the position of the quadrat within the site (interior vs. fringe) could help to quantify the role and speed of lateral recolonization in future management *S. aloides* trials.

Factors such as: treatment method, application timing and varied recovery periods all played a role in determining the treatment impacts on *S. aloides* biomass. The location of the treatment sites was also carefully selected so that interference between treatment methods was not responsible for influencing the biomass and diversity results. In support of this, the fall chemical site (which was down river and the most isolated of any treatment site), had the highest degree of *S. aloides* biomass reductions compared to the other three sites, which were in much closer proximity to one another. The question remains as to why below target *S. aloides* eradication and rapid recolonization was observed in the both the spring/fall chemical and physical treatments.

Two major limitations of this study that may have influenced the results or could have provided more detailed information for their interpretation were that there were no true treatment replicates (pseudo-replicates only) and that there were no untreated *S. aloides* “reference,” sites. At the time this study was conducted, however, there were limited areas of *S. aloides* with the appropriate percent cover, vegetation type, river morphology and distance from other adjacent *S. aloides* patches to add additional replicates. Increasing the number of treatment replicates above once per treatment would help to more precisely identify the causes behind why *S. aloides* biomass fluctuated during this experiment. This could include exposing results that could have been outliers and developing more substantial trends in terms of *S. aloides* response towards herbicide and manual raking. Sampling untreated *S. aloides* reference sites would also help to give a much-improved understanding of how *S. aloides* populations on the Trent River change naturally throughout the year. These results could be used to support increased treatment replicates in answering what changes to *S. aloides* biomass could have been attributed to the treatments alone and how to improve management planning and treatment efficacy. For example, significant *S. aloides* biomass regeneration did not occur between the June 2013 and August 2013 sample periods in the fall chemical site, where as the spring chemical, spring physical and fall physical sites returned to their pre-treatment levels. Since biomass in the other three treatment sites responded similarly to each other, was this result due to an abnormality in treatment conditions or procedures in the fall chemical site, or is *S. aloides* truly more susceptive to fall herbicide applications for reducing biomass?

Considering the results, manual raking was not found to be significantly less effective than the herbicide application for controlling *S. aloides*, however, given the time and labor requirements of this treatment method, it was inefficient for the plot sizes selected in this study (0.25 ha) and is likely not appropriate for *S. aloides* control outside of populations comprised of only a few individuals. The lack of effectiveness of manual raking was related to two factors: i) loss of water clarity and ii) the time/resource requirements for manual control on this spatial scale. Each of these limiting factors is described below.

As plants were uprooted from the river bottom, suspended sediments immediately reduced visibility below the water's surface so that it was impossible to determine if all the plants had been harvested. Even with consecutive days of manual treatment, visual confirmation (from the surface) that all plants were removed could not be achieved at either of the physical control sites (1 and 3). Multiple raking interventions over the same area is generally required for this type of treatment and in extremely dense patches of plants, such as that of *S. aloides* in the Trent River, diver assisted pulling/raking may also be required (Bellaud, 2014).

The sheer size and density of *S. aloides* stands in the Trent River require larger implements than hand rakes operated from small boats to achieve significant biomass reduction. In terms of human resource requirements, the individuals harvesting *S. aloides* in this study spent as much time travelling back and forth from the treatment sites to the shore to dispose of plants as they spent raking the plants. *S. aloides* plants quickly accumulated in the boat and had to be frequently transferred to shore to maintain buoyancy in the watercraft. This greatly reduced the surface area that could be covered and total mass of plants that could be harvested each day. Effective physical management of *S. aloides* on this spatial scale would require larger barge-style boats, mechanical harvesters and an improved method for transferring plants to the shore without the need for the harvester to travel back and forth.

Harvesting efficiency is especially important for species like *S. aloides*, where each individual plant has considerable biomass. Management of the prodigious and densely growing *E. crassipes* in the US using mechanical harvesters shows a great deal of variation in harvesting efficiency. Wolverton and McDonald (1979) examined three different types of harvesters and found harvest rates ranging from low (414 m^2/hr) to high (1670 m^2/hr) that were directly related to the size of the plants being harvested (larger plants resulted in lower harvest rates). Rates of *S. aloides* collection via manual raking during this study have been roughly calculated to 10 m^2/hr, well below the lowest efficiency of mechanical harvesting equipment reported by Wolverton and McDonald (1979). At this rate, harvesting each of the 0.25 ha study sites would take about 250 hours. If one extrapolates this rate to the ~200 ha of *S. aloides* plants that require management in the Trent River and considers the need for repeated harvesting within each area, the time and human resources that would be required make further application of the hand raking method unrealistic.

A secondary factor supporting the regrowth of *S. aloides* in the manual raking stands could be the release/spread of propagules following treatment. Conceivably, offsets could have broken off of mother plants during raking in the spring and both offsets and turions could have potentially been released during the fall raking period. These propagules are likely to descend to the sediment or float outside the quadrat and escape collection by subsequent rakings. Healthy *S. aloides* offsets are able to source nutrients from both the water and the sediment and do not require roots (Mulderij et al, 2005, 2009) allowing for high rates of survival after being dislodged. *S. aloides* turions were shown to have germination rates as high as 60% under ideal conditions (Mulderij et al, 2005, 2009), giving displaced propagules a high chance of development into a mature rosette within the Trent River. Other invasive aquatic plants such as *H.verticillata* and *M. spicatum* demonstrate similar natural defenses against physical control techniques. In these cases, entire plants can regenerate from single leaf whorl fragments broken off and dislodged during physical management activities (Bellaud, 2014; Haller, 2014; Langeland and Sutton, 1980). Turions can remain viable for years until optimal growth conditions are encountered and the plant can reappear (Basiouny et al, 1978). The long-term viability of *S. aloides* turions is currently unknown.

Regrowth of plants following manual control is a common occurrence and results from the literature match those of *S. aloides* regeneration in the Trent River. Similar, fast-growing species have been shown to require harvesting up to six times per year (McGehee, 1979). *H.verticillata* stands that have been controlled using mechanical cutters can regrow back to the water's surface in two months (Haller, 2014). Repeated mechanical removal of *P.stratiotes* between 2003 and 2006 revealed that although harvesting reduced surface coverage of this plant to as low as 1%, regrowth in the following years returned biomass to above 50% coverage each time (Ayala et al, 2007).

Results of physical control techniques reported in the literature are mixed. Harvesting of *Rannunculus spp*. in four rivers in the UK showed that significant regrowth of the plant equal to pre-treatment levels was only encountered in one of the four sites (Fox and Murphy, 1990). At this site, harvesting occurred in a shallow portion of the river where increased light availability was hypothesized as the reason behind the population regeneration (Fox and Murphy, 1990). Regrowth of *S. aloides* plants in the Trent River beyond pre-treatment levels is most likely the result of treatment inefficiency – too many plants remained within the sites following treatments.

The main advantages of using varied seasonal application times to control *S. aloides* are that there are fewer non-target plant species present in the water column in the spring and fall, as opposed to mid-summer, that can be impacted by the treatments and that water clarity is increased (especially in the fall). Potential seasonal impacts on the success of manual raking are nullified by the fact that hand raking is a very selective treatment and impacts to other vegetation are already very low regardless of time of year. Secondly, improved water clarity had no impact on the effectiveness of hand raking, since the suspension of sediment into the water column occurring immediately after upheaval of plant root structures in the spring and fall limited rake operator visibility to a much greater extent than natural seasonal fluctuations in turbidity. In other words, the fact that the water was initially less turbid in fall made little difference; once hand raking commenced, the water became turbid.

Herbicide application showed initial post-treatment biomass reductions slightly greater than those of hand raking, but consistent and extensive regrowth occurred throughout the following sampling periods (**Figure 26**). Diquat has been shown to provide adequate control of *S. aloides* in laboratory settings (Netherland, 2013), yet this efficacy was not evident in the Trent River based upon the failure of chemical applications to meet the minimum 75% biomass reduction target.

Figure 26: *S. aloides* rosette collected from the spring chemical site three months after chemical treatment. Outer leaves of the rosette have decayed (presumably from Diquat damage) to expose two healthy sprouts regenerating from the base of the rosette. Sufficient control from herbicide application was not reached in either the spring or fall treatments

The only previously attempted control of *S. aloides* in the field using Diquat was attempted in 2008 in the Trent River, where the results of the herbicide application were not comprehensively quantified or studied. The lack of information regarding *S. aloides* response to Diquat exposure in field conditions gives little context for explanation of the inability of herbicide treatments in this study to provide acceptable plant control. However, using available literature on treatment response of other submerged aquatic plant species following Diquat exposure, and on the characteristics of Diquat itself, the primary factors responsible for the reduced treatment effect during this study can be deduced.

Diquat treatment success appears to be highly variable based upon plant species and local site conditions. Clements et al (2013) observed similar results as in this study when applying Diquat to *Sagittaria platyphylla* (broad-leaf arrowhead) populations. Minor tissue damage was recorded immediately following Diquat application, but by six weeks after treatment, *S.platyphylla* biomass had exceeded that of pre-treatment levels. This includes treatment failures where the product was considered viable and the application process was sound (Clayton and Matheson, 2010). Even species from the same plant families and genus treated using the same methods have shown differential herbicide response. Glomski and Netherland (2007) found *M. spicatum* to be much more susceptible to Diquat than *M.heterophyllum* (shared Family: Halogoraceae and Genus:

Myriophyllum). This was observed between *H.verticillata* and *E. canadensis* (Family: Hydrocharitaceae) where Diquat was much more effective on *E. canadensis* (Glomski et al, 2005).

Determining the factors responsible for Diquat deactivation and/or loss of treatment efficacy varies between each application, but factors for reducing the effectiveness of Diquat on *S. aloides* in the Trent River appear to be: i) herbicide-plant exposure time ii) herbicide blocking aufwuchs and particulates, iii) water temperature, iv) light/photosynthesis-Diquat interactions, vi) application method. Each of these factors will be discussed below.

i. Herbicide-plant exposure time

Herbicides require minimum periods of exposure time with the target plant to produce consistent control. This is called the concentration/exposure time (CET) relationship. Depending upon the herbicide and its method of action CET can range from several hours for contact herbicides to many days for systemic chemicals (Getsinger et al, 2011). Diquat is generally considered a fast acting, contact herbicide, requiring exposure times of between 6-36 hours for effective control of submerged aquatic plants like *M. spicatum*, *H.verticilata* and *E. canadensis* (Getsinger et al, 2011; Glomski and Netherland 2007; Glomski et al, 2005). A previous laboratory-based efficacy trial indicated that exposure time of between 8-48 hours was sufficient to allow for significant biomass reduction of *S. aloides* rosettes, but full plant death only occurred in temperatures above 20°C, regardless of exposure time (Netherland, 2013). In that study, plants were treated in closed containers and static systems where herbicide dispersal due to water velocity was not a factor.

Diquat application control is more severely limited in flowing systems, where water exchange can push the herbicide outside of the treatment area, especially when applied in spot or small-scale treatments, which may lower the CET relationship beyond effective limits for the target species (Fox et al, 1991; Getsinger et al, 1996; Skogerboe et al, 2006). Skogerboe et al (2006) observed that water samples of Diquat concentrations in simulated flowing water systems were 14 to 25% less than target application rates only 30 minutes after application. Ritter et al (2000) reproduced water conditions representing six areas of the US in a laboratory-based study of Diquat uptake that reflected lake, canal and farm pond conditions. Factors such as the presence of sediment, dispersion, water velocity and plant biomass were accounted for in the model. While Diquat concentrations dissipated most rapidly in lake conditions, even in the closed-system pond scenarios the dissipation rate was described as very rapid. Chemical binding of Diquat to plant and sediment was reported as greater than 99.9% complete within hours of application (Ritter et al, 2000).

Getsinger et al (1990) recorded the effect of flowing water on herbicide concentrations applied to submerged plant beds in the field. In these studies, flow rates as low as 1 cm/second were sufficient to cause disruptions in herbicide contact time and efficacy (Getsinger and Westerdahl, 1986; Getsinger et al, 1990; Getsinger et al, 1996). A limitation in the present study was that no flow rate measurements were taken before the herbicide was applied to the treatment sites. The main channel of the Trent River is under constant flowing conditions due to wind, current and the opening and closing of navigation locks that control water levels and flush huge volumes of water through the system. From anecdotal observations (without taking quantitative measurements), flow rates in the Trent River are likely well above the 1 cm/sec velocities needed to disrupt CET relationships and may be responsible for reducing Diquat contact time below minimum requirements.

Velocity effects on herbicide dispersal are increased when applied to small plot treatments (Skogerboe et al, 2006). Small treatment areas (between 0.5 and 2 ha) have been shown to reduce herbicide-plant exposure, with sites over 5 ha allowing for the longest exposure times (Netherland, 2013 per. comm.; Poovey et al, 2004). The treatment sites used in the present study were 0.25 ha in size, which falls well below the ideal size for maximizing contact times, especially considering the dispersal effects caused by flowing water. Having said that, the practicality of using large treatment areas (over 5 ha) in a study such as the present one, would pose other challenges, particularly in relation to the hand raking treatment.

Future herbicide trials would likely benefit from application over larger areas than were tested in this study. However, the distribution of *S. aloides* within the Trent River at the time of this research limited the size and number of areas that could be tested without increasing the risk of interference between herbicide treatment areas. Site sizes used in this study were maximized based on the spatial scale of the invasion at that point in time. At the current time, *S. aloides* distribution on the Trent River is much more extensive than it was in 2013; the use of larger treatment plots may now be feasible.

ii. Herbicide blocking aufwuchs and particulates

Coupled with CET relationships in the Trent River, water turbidity and the presence of particulate matter on the plants could have also reduced the effectiveness of

Diquat in the current study. Suspended sediments and aufwuchs have been identified as major factors that need to be considered when using chemical control techniques (Glomski et al, 2005; Hofstra et al, 2001; Netherland, 2013; Poovey and Getsinger, 2002), although these results vary. In waters where turbidity is high, the effectiveness of contact herbicides may be diminished due to binding with suspended particulate matter. Dissipation rates of Diquat due to suspended solids have been measured at between 11 and 96% after 30 minutes of exposure (Skogerboe et al, 2006; Yeo, 1967). Diquat has shown good control of *Egeria densa* in low turbidity waters (5-15 NTU), but virtually no effect above 25 NTU (Poovey and Getsinger, 2002). Conversely, Diquat was proven ineffective at *C. demersum* control in waters where turbidity was equal to or above 10 NTU (Hofstra et al, 2001). Similarly, Diquat was completely deactivated by clay particles in Australian irrigation canals where turbidity ranged from 10-80 NTU (Bowmer, 1982a).

With respect to *S. aloides*, Netherland (2013) showed that Diquat was able to reduce rosette biomass at all turbidity levels used in a laboratory study (0-16 NTU). Herbicide concentrations below 180 μg/L or exposures fewer than 8 hours were identified as factors that would likely reduce Diquat efficacy in turbid waters. The selected turbidities of 0-16 NTU represent fairly low levels of suspended particulates and Netherland's (2013) study did not fully explore the impact of high turbidity levels (e.g., 25 NTU’s and above) although these levels are not likely to be encountered on the Trent River. A second limitation to the current study is that no measures of turbidity were taken at the treatment or reference sites, although the Trent River is generally considered of moderate to high water clarity and would likely fall into the low turbidity category of 0-15 NTU.

Similar to the effects of turbidity, the impact of excessive aufwuchs on the plants has also been shown to reduce Diquat uptake (Bowmer, 1982b). The presence of sediment and other particles on the surface of leaves presents a physical barrier that reduces the area available for uptake of a contact herbicide such as Diquat. Aufwuchs may also bind with Diquat and reduce the amount of herbicide available to bind with the plant. *S. aloides* plants grown under experimental conditions in Florida, as well as within the Ontario population, tended to support relatively high proportions of aufwuchs (Canning, 2012-2015 per. obs.; Netherland, 2013). The presence of these substances has been shown to be responsible for 8 to 44% deactivation of the active ingredients in Diquat and was found to be a substantial barrier to use of this herbicide for *Lagarosiphon major* control (Clayton and Matheson, 2010). Of particular note in the Clayton and Matheson (2010) study was the finding that deactivation of Diquat was significantly higher during the summer as opposed to spring and fall applications. With the treatment periods in the present study confined to the spring and fall, it would appear that the impacts of aufwuchs would have been lessened at those times. Additionally, it is primarily the older, outer leaves of *S. aloides* that are most heavily colonized by aufwuchs. Netherland (2013) suggested that since *S. aloides* lacks multiple meristems that can facilitate recovery, the exposed and aufwuchs-free basal meristem of the plant is likely to remain susceptible to Diquat treatments despite the presence of particulate matter on outer leaves (Netherland, 2013).

iii. Water temperature

The impact of water temperature on Diquat efficacy towards *S. aloides* control is two-fold. Netherland (2013) demonstrated the role of temperature on the efficacy of Diquat towards reducing *S. aloides* biomass by applying herbicide to plants in separate tanks containing 15°C, 20°C and 25°C water. Regardless of herbicide rate or exposure time, regeneration of *S. aloides* tissue was evident 21 days after treatment in the 15 and 20°C water, but plants were completely destroyed following application in 25°C water with no tissue regeneration (Netherland, 2013). Optimal temperatures for Diquat uptake are likely related to rates of photosynthesis in plants because Diquat requires the active source of electrons from photosynthetic processes to maximize toxicity (Hess, 2000). Rates of photosynthesis have been seen to change in response to temperature variations, with the highest rates generally occurring between 14-35°C (Hyldgaard et al, 2014; Santamaría and van Vierssen, 1997). This suggests that applying Diquat in mid-late summer, when ambient temperatures are well above the 25°C threshold, could help to optimize Diquat effectiveness in the Trent River. Netherland (2013) hypothesizes that the use of higher treatment rates and longer exposure times might counteract the impact of lower water temperature on Diquat effectiveness.

It is of interest to note that on the U.S. label for Diquat (Reward®), application of this product is not recommended for water temperatures below 10°C (Syngenta Crop Protection LLC, 2014), but no such restrictions are present on the Canadian version of the label (Syngenta Canada Inc, 2015). Regardless the source of this discrepancy, temperatures recorded at the treatment sites during the Diquat applications were 18°C in the spring and 15°C in the fall and were well within the specified limits for this herbicide.

iv. Light/photosynthesis-plant growth form-Diquat interactions

As a photosystem 1 inhibiting herbicide, Diquat is primarily dependent upon electron transport chain disruption to cause cell membrane and tissue degradation of the target plant (Hess, 2000; Mees, 1960). The most readily available source for electron uptake by Diquat is from irradiation driven biological processes, primarily, photosynthesis (Funderburk and Lawrence, 1964; Hess, 2000). Indeed, light availability/increased rates of photosynthetic activity have been shown to improve the desired effect of Diquat (Mees, 1960). It can be hypothesized then that Diquat application will provide optimized control results for healthy *S. aloides* plants, in full light conditions, which are experiencing high rates of photosynthesis.

In the literature, Diquat toxicity has been observed to some extent in the dark (Hess, 2000; Jordan et al, 1966; Mees, 1960), but nowhere near what is encountered under irradiant conditions (Hess, 2000; Mees, 1960). In these cases, respiration was identified as the source of electrons for Diquat uptake during periods of no light exposure, but the supply of available electrons in these cases was much lower than during photosynthesis (Hess, 2000; Mees, 1960). Jordan et al (1966) studied the effect of seven photosynthesis-inhibiting herbicides on *Nicotiana tobacum* (tobacco) tissues that were treated and left in dark conditions for 21 days. They found that paraquat (the tested herbicide most similar to Diquat), was able to cause plant death at rates above 10^{-6} molar herbicide concentration even under fully dark conditions (Jordan et al, 1966).

Another factor to consider related to the role of light/photosynthesis promoting/inhibiting Diquat toxicity of *S. aloides* is plant growth stage and leaf form. Submerged aquatic plants are commonly reported as having significantly lower photosynthetic rates than that of terrestrial species (Binzer and Sand-Jensen, 2002a; Nielsen and Sand-Jensen, 1989; Sand-Jensen, 1997; Sand-Jensen et al, 2007). This is primarily related to the fact that photosynthesis is associated with chlorophyll concentrations in leaves (Nielsen and Sand-Jensen, 1989). Terrestrial plants have thicker leaves, with more surface area than submerged macrophyte species and therefore, can contain greater concentrations of chlorophyll, which increases photosynthetic output (Nielsen and Sand-Jensen, 1989).

S. aloides is unique among macrophytes in that it can undergo multiple growth form changes throughout the year and has different leaf properties depending upon whether the material comes from a submerged rosette, emergent rosette, germinated turion or offset etc. Nielsen and Borum (2008) measured photosynthetic rates among four different *S. aloides* leaf types and found significant rate variations depending upon chlorophyll content as a function of specific leaf area. Recently germinated turions were found to have the thinnest leaves. Emergent rosettes, which had the thickest leaves and developed stomata, were three-five times more photosynthetically active than their submerged counter parts (Nielsen and Borum, 2008). Considering Diquat uptake, for which higher rates of photosynthetic activity have been linked to increased plant toxicity (Hess, 2000; Mees, 1960), it would appear that emergent leaf parts would be more receptive to herbicide treatment than any other leaf form.

v. Herbicide application methods and labeling

The presence of a single meristem in *S. aloides* plants presents a potential weakness that could be exploited by appropriate application of Diquat. As such, Netherland (2013) states that it is critical that the basal meristem be exposed to Diquat for treatment to be effective. In the present study, Diquat was applied using a wand that dispersed the chemical under the water surface and larger scale treatments by the OMNRF have employed the use of surface boom sprayers. The extent to which the herbicide was able to make contact with the meristems of target plants is not known in either of these instances.

Laboratory and greenhouse trials on Diquat have shown that this herbicide is sensitive to several environmental factors that impact efficacy. These primarily being: turbidity, flow rates and temperature (Fox et al, 1991; Getsinger and Westerdahl, 1986; Getsinger et al, 1990; Glomski et al, 2005; Hofstra et al, 2001; Netherland, 2013; Poovey and Getsinger, 2002; Skogerboe et al, 2006). These same parameters have not been assessed or studied when considering field application of Diquat to *S. aloides* populations and remain unknown variables in Diquat treatment success in the Trent River. If these variables (flow, turbidity and temperature) serve as significant limiting factors to Diquat toxicity in laboratory experiments, these impacts should also be appropriately quantified in the context of real site conditions encountered on the Trent River.

In regards to this, a limitation of this study was the inability to exercise full control over spraying procedures, herbicide inhibiting parameter measurements or to verify the method in which the herbicide was administered. Classical experimental design is based upon the precise manipulation of controlled experimental variables to identify factors that are directly influencing the research question or driving the phenomenon that prompted study in the first place. Without such control, it is impossible to definitively identify the underlying factors behind the observed results.

The results of this study on *S. aloides* response to hand raking and chemical control and seasonal treatment times can be used to inform recommendations for adaptive changes to current management methods to improve the levels of control. In terms of physical control methods, larger scale, high capacity mechanical equipment is required for efficient control of this plant and hand pulling should only be employed for small numbers of plants in shallow water.

3.4.2 Treatment Effects on Existing Plant Communities

A small number of statistically significant reductions in existing plant biomass were recorded across sampling periods and between the treatment and reference sites. These fluctuations in existing plant biomass were short lived and were not maintained between sample periods. The largest increase in local community biomass occurred during the August sampling period in the fall chemical site, which corresponded with the sustained reductions in *S. aloides* biomass seen in that site between June and August. This could indicate the potential for quick recovery from existing plant communities, given that sufficient *S. aloides* biomass is removed to inhibit its population regeneration through the season. In all sampling periods, species richness and SWI in the treatment sites was statistically equivalent to the measured values in the reference site and suggests that these study parameters were tolerant of both *S. aloides* presence and treatment disturbances.

The local vegetation sampling provided an unexpected result in that both the biomass and diversity of the local plant communities growing amidst the *S. aloides* treatment sites was equal to that of the reference site. This is surprising because based upon the literature and observations from the Trent River, *S. aloides* is well known as a prolific, canopy forming, monoculture promoting species. Given its invasive and competitive tendencies, how were local communities growing with *S. aloides* able to maintain equivalent biomass and diversity levels as the reference site, even during the pre-treatment assessment? Manual raking provided a much higher degree of selectivity in what plants were removed compared to the herbicide, which impacts all species present. It was assumed then, that biomass and diversity within the manual raking sites would have been greater than that of the chemical sites. Evidently, this was not the case as both control types were found to be equal based upon those parameters and comparison to the reference site.

The scope of this experiment cannot fully explain this occurrence, however the similarities in biomass could be explained by the great increase in *C. demersum* within the treatment sites, which would have helped bolster biomass results even as other plants declined due to the presence of *S. aloides*. The treatments sites were selected for as close to 100% *S. aloides* cover as possible (~75-90%), which suggests that *S. aloides* populations may require higher densities than what was studied here to more significantly inhibit local plant growth. A potential trade-off for selecting sites with nearly 100% cover of *S. aloides* seemingly could have been that the local communities were already suppressed by *S. aloides* and were in a state of disturbance before the sampling. In that case, perhaps it was the reference site that was abnormally low in biomass and diversity at the time of sampling? It seems possible that the treatment methods did not remove sufficient quantities of *S. aloides* to precipitate structural changes within the plant communities in the sample sites, which could have been why no changes to richness and

SWI were observed. Indeed, by looking at the different plant species recorded within the treatment sites the treatment did not encourage colonization by new species, but rather caused already existing species to increase or decrease in biomass.

Alternatively, evidence from *S. aloides* populations in its native range indicates that this species actually promotes biodiversity (Strzałek and Koperski, 2009; Sugier et al, 2010) and supports a greater range of insect and bird species compared to other vegetation types (Rantala et al, 2004; Suhonen et al, 2013; Van der Winden et al, 2004). Its ability to promote biodiversity has not been explicitly related to other plant species, however, it appeared that *S. aloides* was either not able to reduce or actually promoted the biomass/diversity of local plants at least equivalent to that of reference site levels.

As outlined in section 3.4.1, there was an 8 month time difference between when the fall sites were treated and then sampled as opposed to the spring sites, which were sampled within weeks of their chemical and physical treatments. This had no discernable effects on *S. aloides*, but may have impacted local plant communities as the fall chemical site had higher biomass in the fist post-treatment sapling period than the reference and spring chemical sites. This suggest that perhaps fall treated local plant communities were able to recover to a greater extent given the increased time between treatment and sampling, although the fall physical site did not show the same high level of local recovery as in the fall chemical site. In this case, was the increase in fall chemical local plant biomass in June 2013 due to increased Diquat uptake, the temporal advantage those plants gained or some other combination of factors?

The results provided by this experiment are not able to fully describe why changes to local plant biomass and diversity did or did not happen in relation to the presence of *S. aloides*, response to control measures and compared to the reference site. Sampling only one reference site and having only a single treatment replicate was a limitation of this study, which made interpretation of the results difficult. Increasing the number of treatment replicates and including more than one reference site in the future could help to provide a more detailed analysis of the results and pinpoint what drove changes to the local plant community throughout the duration of this experiment.

Overall, these findings support the lack of success achieved by the *S. aloides* control treatments. Minor fluctuations across the sampling period are most likely the effects of environmental influences (seasonal changes, overwintering etc.) more so than direct impacts of the control treatments. The significant decreases in *S. aloides* biomass that would be required to allow for biomass/diversity shifts did not occur within the sample areas and the plant communities within those sites represented fairly stable systems over the course of the study. Long-term, significant reductions in target plant biomass are required to promote the recovery of any stable, local vegetation communities (Tanner et al, 1990; Tanner et al, 1990a).

Invasive aquatic plant treatments that are deemed successful are associated with some type of response related to existing plant biomass or diversity. Changes to the local plant communities after treatment were generally noticeable within a short period of time (~ one month minimum), however full recovery of submerged systems to their pre-invasion/historical states can require years to achieve (Klein and Verlaque, 2011; Ogdahl and Steinmann, 2014). Johnston et al (1983) used manual control, herbicide and *C.idella* (grass carp) to eradicate a monoculture *H.verticillata* population from a 0.25 ha pond. Within eight months following treatment, *Nelumbo lutea* (American lotus) had colonized

20% of the open water areas and eventually reached 90% coverage. At the end of the three year study period, twelve species of macrophyte were identified in the pond and no regrowth of *H.verticillata* was recorded, which was attributed to increased competition from the newly established plant species (Johnston et al, 1983). Xu et al (2014) manually removed floating mats of *Trapa bispinosa* (Water chestnut) from Lake Taihu in China in an effort to restore the submerged macrophyte community. At sites where 50-100% of the *T. bispinosa* plants were removed, a significant increase in biomass growth of *M. spicatum* was observed after only 20 days and levels increased following continued *T. bispinosa* harvesting. In areas where *T. bispinosa* harvesting did not reduce the target plant's biomass below 50%, no significant increases in *M. spicatum* biomass occurred (Xu et al, 2014). Torn et al (2010) simulated human disturbances by removing varying amounts of lake sediment to determine the response of aquatic plant communities. Within two months, the study plots were fully recolonized by *Najas marina* (Spiny water nymph) and minor to moderate sediment disturbances were shown to allow for fast recovery by aquatic plant ecosystems (Torn et al, 2010).

At the opposite end of the spectrum, unsuccessful management actions that caused minimal or temporary impacts to the target plant species showed no improvement in local community biomass or diversity following treatment, mainly due to the recovery of the invasive plant (Fox and Murphy, 1990; Kaenel and Uehlinger,1999; Unmuht et al, 1998). Bickel and Closs (2009) used mechanical cutters and divers to remove all above ground biomass and residual stem material of the dominant invasive *L.major* to form three plant free channels within a study lake. Four months after treatment, *L.major* had recolonized 75% of that area. Increased light and nutrient availably after the removal of plant material was actually seen to support encroachment from adjacent *L.major* patches. Coverage of competing native and invasive species within the lake never exceeded 5% throughout the study period (Bickel and Closs, 2009). This might indicate that competing native species did not have sufficient time to recover before recolonization by *L.major* occurred and that this species was better suited to colonization these disturbed sites.

In some cases, environmental factors such as propagule bank disturbance and high turbidity have been seen to prevent or delay the recovery of aquatic plant communities (Ayala et al, 2007; Ogdahl and Steinmann, 2014), but the removal of a dominant invasive species generally resulted in abundance shifts within the ecosystem. Lack of response to herbicide or manual controls on either *S. aloides* populations or existing plant communities is a sure indicator that the treatments used in the present study were not effective. Manual raking is likely to pose minimal impacts to non-target plants in the first place, but as a non-selective herbicide, the effects of a successful Diquat application should have been much more clearly evident on other plants. With such minimal reductions in *S. aloides* biomass, it appears that competing plants in the system were not able to increase their diversity or abundance within the Trent River and that threshold values for reductions in *S. aloides* biomass to allow for native plant recovery are well above the 47-73% observed in the present study.

3.4.3 *S. aloides* Species Assemblages and Post-Colonization Macrophyte Community Dynamics

Dominated by dense, floating *S. aloides* stands since 2008, macrophyte communities in the Trent River have likely begun adapting to the presence of this light limiting species. The frequency of occurrence of species in quadrats with *S. aloides* indicates that certain species associations may have developed in response to *S. aloides* colonization (**Table 5**), specifically the relationship between *S. aloides* and *C. demersum*. This species was most often found growing with *S. aloides* compared to all other macrophytes in the system and typically had the highest biomass in samples (after *S. aloides*). In the reference site, the presence of *C. demersum* was lower in both occurrence and biomass than in the treatment sites where *S. aloides* was present. Instead of *C. demersum*, the reference site had increased occurrences of *V. americana* and *E. canadensis* in June and the invasive specie's *M. spicatum* and *P. crispus* in the fall. This suggests that *S. aloides* was able to inhibit the success of or displace these invasive plants in the treatments sites and that certain aspects of *S. aloides* colonization created conditions favorable for increased *C. demersum* growth. Sampling only one treatment and reference plot replicate was a limitation of this study, which in future experiments, could be increased to provide more evidence to support community dynamics trends. Given this limitation, *C. demersum* has been shown to frequently co-habitat with *S. aloides* in its native range (Smolders et al, 1996c, 2003; Smolders and Roelofs, 1995).

The growth of *C. demersum* in association with other invasive aquatic plants has been documented and appears to be a result of light limitation by densely growing, floating or canopy forming species (Lougheed et al, 2001). Macrophytes able to form surface canopies have been shown to not only increase their uptake of PAR, but to increase shading and inhibit the growth of competing plant species (Barko and Smart, 1986; Sculthorpe, 1967). In these instances, submerged, low light tolerant species are found to be growing successfully as opposed to similar canopy forming plants with high light requirements. *C. demersum* can grow under fully established plant canopies (Lacoul and Freedman, 2006), similar to conditions present on the Trent River in areas where *S. aloides* has established expansive emergent colonies.

C. demersum is well suited to growing under *S. aloides* populations as it is tolerant of both low light and turbid conditions (Lougheed et al, 2001). Its non-competing growth form (low light tolerant, submerged vs. high light requirement, canopy forming/floating) occupies a different functional niche in the water column than *S. aloides*..

Ginn (2011) assessed macrophyte communities in Lake Simcoe, ON and found that *M. spicatum* dominant communities colonized water depths above 3.5m, where as deeper than that, *C. demersum* was typically the only species present (Ginn, 2011). Hofstra et al (1999) studied the growth of *H.verticillata* in the presence of four other competing macrophytes: *C. demersum*, *E.densa*, *L.major* and *E. canadensis*. *C. demersum*, the species most tolerant to low light and the least morphologically/physiologically similar of the four, showed no decrease in biomass in association with the presence of *H.verticilatta*. Instead of competing against *H.verticillata,* the presence of *C. demersum* prompted increased growth and tuber production in the target species where as the other competitors, *E.densa*, *L.major* and *E. canadensis*, did not (Hofstra et al, 1999). As a result of *H.verticillata*, *E.densa*, *L.major* and *E. canadensis* competing for space at the top of the water column, *C. demersum* was able to occupy a niche at the bottom of the system and was the only plant not to suffer any negative growth effects from plant competition.

The identification of *C. demersum* as the macrophyte most frequently associated with *S. aloides* in the Trent River, supports findings from Chapter 2 that the plant species best able to co-exist with *S. aloides* must be well suited to surviving in low light conditions related to both water column light attenuation and vertical shading from other macrophytes. After comparing the light requirements of other species found in Ontario (**Table 2)**, it becomes evident as to how *C. demersum* is able to thrive under these conditions. The macrophyte species with the lowest light compensation points after *S. aloides* are *E. canadensis* (3-17.8 µmol $m^{-2}s^{-1}$) and *C. demersum* (7-35 µmol $m^{-2}s^{-1}$). As outlined in Chapter 2, a lower compensation point value can allow a plant to continue photosynthesizing at lower light intensities and increases its maximum depth of colonization. In order for a species to survive in the light limited conditions under *S. aloides* populations, it must be physiologically adapted to low light environments like *E. canadensis* and *C. demersum*. Although *E. canadensis* has a lower range of compensation point values than *C. demersum*, this species is not nearly as abundant in the Trent River, which could explain why it did not form the same type of functional relationship with *S. aloides*. Additionally, as a species that has a tendency to occupy the upper reaches of the water column and become canopy forming, it may have been out competed and replaced by *S. aloides* prior to the monitoring periods in this study. Spencer and Ksander (2001) also noted this relationship between light compensation and plant community establishment. Macrophytes with lower light compensation values were able to dictate how plant communities were formed by outcompeting and displacing plants not adapted to lower light environments.

The relationship between *S. aloides* and *C. demersum* suggests that instead of competing against one another, they are able to co-exist and can mutually support their own success. One aspect of invasive species management relies upon competition between species to help displace undesirable vegetation (Johnston et al, 1983), which in this case, has seemingly to be achieved given the current macrophyte community in the Trent River.

CHAPTER 4: Conclusions

The results of this study help to fill key knowledge gaps concerning the biological capabilities of *S. aloides* and its response to management actions in the Trent River. At the same time, study findings reveal areas where further research is required and identify more questions that need to be answered to inform the continual development of Ontario's *S. aloides* IMP (OMNRF, 2013). The findings of the treatment component of this study have implications related to herbicide efficacy under field conditions, understanding Diquat effects on *S. aloides* propagules, testing alternate methods of physical/mechanical control, and re-evaluating management outcomes.

The role of light as a primary limiting factor for macrophyte growth in the Trent River was explored and light requirements for both types of *S. aloides* propagules (offsets and turions) were assessed (Chapter 2). Turions were able to germinate under the full range of tested light intensities, which were equivalent to approximately 22.0 to 0.3% of surface light in the river, however, they are unlikely to survive after germination under the lowest studied light intensities. Offsets were suspended throughout the water column to measure their net rate of photosynthesis at attenuated light intensities and were able to maintain active photosynthesis until ~5m in depth. Using a linear regression model, the light compensation points for *S. aloides* offsets (where net rate equaled 0) were calculated to be between 5.25 and 5.39 m. The maximum depth of colonization for offsets was estimated using the compensation point and was determined to be between 4-6m. Since *S. aloides* propagules tolerated a wide range of light intensities and water depths, the potential of these reproductive units to continue to establish new genets in almost all areas of the Trent River becomes evident. The quantification of a Z_c value for *S. aloides* in the Trent River can help to inform management by identifying water bodies that could be susceptible to colonization by this plant and should focus monitoring into areas where water depth does not exceed 6m.

Given this growth capacity, future research needs to be directed at the dispersal characteristics of propagules specifically related to informing *S. aloides* monitoring and management. How long do turions remain viable after being released from the plant? How far can the current carry them within this waterway? Are propagules accumulating in certain areas downstream based on water flow or are they randomly distributed throughout the system? Is there a role for the TSW locks in preventing *S. aloides* spread? It is clear that these plants can survive in Ontario; attention must now be directed at how they are moving throughout suitable waters within the province.

The photosynthetic capabilities of *S. aloides* appear to be related not only to directing monitoring effects and predicting spread, but also in improving the effectiveness of chemical treatments. This research has exposed many unanswered questions in terms of widespread *S. aloides* management. Since Diquat does not translocate throughout a plant, would application to only emergent leaf parts be sufficient to cause tissue destruction of the submerged basal meristem? In chapter 2, photoinhibition was observed in offsets exposed to full sun. For offsets connected to mature, emergent plants that have not developed sun adapted leaves, could it be possible for Diquat to kill the mother plant, but not the propagules? Does Diquat impact attached, ungerminated turions? Is Diquat less effective in low light and deep-water areas under field application conditions? Can threshold Diquat concentration values sufficient to cause plant death be determined for a range of light intensities, depths and leaf types? Is there a measurable interaction occurring between Diquat toxicity and varying photosynthetic activity of *S. aloides* leaf types and ecomorphs?

Considering that *S. aloides* has a relatively low light compensation point (as established in Chapter 2) and can survive at depths below what might be expected of other macrophytes, is there a threshold PAR/photosynthetic rate/water depth value in which *S. aloides* plants can survive, but do not maintain high enough levels of photosynthesis to be affected by the herbicide? What increased concentrations of active Diquat ingredients would be needed to kill S. aloides plants in conditions, which due to light attenuation throughout the water column are nearly fully dark? Furthermore, how would these concentrations be applied and maintained at lower depth limits and within expansive genets, where such conditions would be encountered? If large-scale management of *S. aloides* in the Trent River is to be carried out using chemical treatments, the answers to these questions may result in the difference between partial/ineffective control and full eradication.

The effects of physical and chemical treatments when applied in the spring and fall for *S. aloides* eradication and local plant community recovery were also described (Chapter 3). Manual raking and Diquat applications used in this study had no significant lasting effects on *S. aloides* biomass reduction. Post-treatment vegetation sampling showed that *S. aloides* populations had recovered to equal or greater levels of abundance than what was measured in pre-treatment sampling by the end of the growing season. The mass of *S. aloides* plants and the density in which they are present, coupled with the loss in visibility after disturbing the sediment while uprooting the plants, made using manual raking not logistically feasible or effective for treatment plots of this size (0.25 ha). River flow rates and application over small plot sizes negatively impacted the CET relationship and reduced the efficacy of Diquat on *S. aloides*. Yet, it has been proven in a laboratory setting that Diquat is an effective means of *S. aloides* control (Netherland, 2013).

What steps need to be taken to achieve the same results in a field setting? Use of a larger treatment plot size (over 5ha) may be needed to adequately measure the impact of Diquat application on *S. aloides* in the flowing waters of the Trent River. The presence of suspended particulate matter in the water and aufwuchs on *S. aloides* leaves created barriers that reduced Diquat's availability for uptake by the plants. Can these losses be quantified? How can Diquat application be adapted to account for these losses? Diquat is recommended for use in flowing water due to its (relatively) low contact time, yet the recommended application rates do not take water flow into account. Can water flow rates be reduced to improve Diquat uptake? Should application rates be adjusted not just for water depth, but also for water velocity? What are the abilities of herbicide adjuvants such as dyes and penetrates to increase efficacy of Diquat uptake in conditions similar to those in the Trent River? What are the roles of plant density and turbidity in limiting Diquat uptake? Are turions susceptible to herbicide treatment? Diquat treatment efficacy in the Trent River could likely be improved by:

- measuring flow rates and turbidity levels prior to application and adjusting treatment rates accordingly
- using a florescent dye such as rhodamine WT to provide better indications of water movement and chemical displacement throughout the test sites
- ensuring that Diquat is applied in a manner that targets the plants meristems
- applying Diquat during periods when temperatures exceed 25 degrees C
- using herbicide adjuvants, such as penetrants and drift reducers, to help to reduce chemical movement away from the target areas in moving water and improve uptake of Diquat through the thick leaves of *S. aloides* (Ferrell, 2014; MOE, 2009)
- increasing treatment site size above 0.25 ha to improve CET relationships on the Trent River, which are likely a key factor in determining the success of Diquat treatments.

With no permanent decreases in *S. aloides* biomass after physical or chemical treatment, local plant communities were unable to recover and increase in abundance in the treatment sites. Would a sustained chemical treatment regime (2-3 years) achieve more lasting results? Can the natural loss (~40%) of biomass experienced by *S. aloides* over the winter season be exploited as part of a longer-term eradication strategy?

Although species composition was slightly different among the treatment sites, biomass and diversity of the local vegetation communities growing in those plots were not significantly different compared to the reference plot. The frequency and abundance of the low-light tolerant species, *C. demersum*, increased under the light limiting *S. aloides* canopies and formed the most significant association with this plant. In comparison, the presence of *C. demersum* in the reference site was much less pronounced and these communities were dominated by high-light requirement species such as *M. spicatum*. If *C. demersum* represents the most abundant species in the Trent River after *S. aloides*, but is also the most likely to support *S. aloides* growth, will *C. demersum* dominant communities be sufficient to prevent recolonization by *S. aloides* following successful plant management? In this case, is there benefit in selecting species better suited for directly competing against *S. aloides* than *C. demersum* and supplementing their populations in the Trent River through over-seeding and propagule planting following management actions?

The results of this study might also make one question whether or not eradication is a realistic target for *S. aloides* in Ontario. The Aquatic Plant Management Society Inc. (APMS) defines viable plant control as, “techniques used alone or in combination that result in a timely, consistent and substantial reduction of a target plant population to levels that alleviate an existing or potential impairment to the uses or functions of the water body” (APMS, 2016). The 2014 Ontario IMP for *S. aloides* indicates its primary management goal as eradication by causing >99% reduction in target plant biomass

(OMNRF, 2013). Based on those parameters, the present research failed to reach the goal of *S. aloides* control in the Trent River.

The development and implementation of a functional IMP is an extremely complex process. It involves a multitude of variables including environmental factors, plant biology, target system ecology, economic issues, and human values (Havel et al, 2015). The introduction and subsequent management of *S. aloides* in Ontario can be used as a clear example of this complexity. Funding limitations, time constraints, biological knowledge gaps, plant resistance to treatment and the scale of the invasion complicate our ability to achieve the optimal outcomes set out for this species in the 2014 IMP using chemical and/or physical treatments.

The literature examining long-term impacts of aquatic plant eradication programs is varied. In some cases, native species have regained dominance over time, while in others, invasive species continue to flourish once control measures are ceased (Havel et al, 2015). The removal of one invader can also promote the success of another (Havel et al, 2015). Cases where full eradication of an invasive aquatic plant took place are relatively few. Situations where invasive species could be effectively isolated from native communities to allow for concentrated treatments without substantial non-target impacts report the highest degree of success (Havel et al, 2015). In the Trent River, the likelihood of isolating *S. aloides* plants from native species is low, since existing plants have developed functional relationships with *S. aloides* and live in close proximity with one another.

The current management plan could be adapted to reflect key elements of successful eradication programs such as: multi-year commitment to control measures, funding support for high cost per hectare treatments and maintenance of long-term monitoring programs (Netherland and Schardt, 2014). Adjusting current outcomes to fit with available resources, public ideals/tolerance for aquatic plant colonies and local site conditions (Netherland and Schardt, 2014) may reveal that maintenance control (~70% biomass reductions) should take priority over eradication as a more realistic goal for the widely established populations of this species on the Trent River.

The broader impacts of this study point to the importance of prevention as the most important tool ecosystem managers have for restricting the impact of *S. aloides* in Ontario. It is clear from Chapter 2, that this species is capable of surviving in portions of, if not all, habitable areas within most water bodies in Ontario. Given this, it seems increasingly feasible to focus resources on spread/introduction prevention for limiting and dealing with this species in its current location.

Preventing new or future invasions is more effective than any known control method for dealing with invasive aquatic plants after their introduction (Havel et al, 2015; OISSP, 2012). This plant demonstrates a high degree of survivability and competitiveness in Ontario waters and the morphological characteristics of the Trent River appear to increase treatment difficulty and reinforce the plant's success.

The first objective of the Ontario *S. aloides* IMP is prevention of the introduction and spread of this species, which is commonly identified as the most important aim in other invasive species management plans (OMNRF, 2013). Most of the current management actions for *S. aloides* are treatment responses to already existing populations, while pro-active measures for prevention take a secondary position. If available control techniques are not meeting established outcomes for *S. aloides* eradication, perhaps more resources should be redirected to emphasize prevention activities. Methods for the prevention of future introductions of *S. aloides* populations could include:

- Incorporating a budget into the Ontario IMP that identifies the methods and costs of prevention and educational programs
- Developing legislation that provides resource managers with legal tools for enforcing the restriction of sale, transport and possession of *S. aloides* within Ontario
- Integrating public education and monitoring of *S. aloides* with current technological trends to include smart phone apps and websites etc., that could help to target and inform a wider range of audiences about this species
- Increasing efforts for *S. aloides* public education in high risk and concentrated traffic areas such as the TSW locks, marinas and boat ramps, as well as areas predicted to be highly at-risk for new genet development
- Focusing resources on research relating to transmission pathways and dispersal mechanisms of this plant

Physical means of preventing further *S. aloides* spread from established populations in the Trent River could include:

- Installing temporary or permanent barriers around *S. aloides* patches to stop water based movement of mature rosettes (potentially turions/offsets as well) and disturbance of plants by recreational traffic
- Installing barriers or filters above and below locks to stop rosette and propagule transmission into new sections of the TSW. Where secondary river diversions/outputs exist (i.e. water falls, feeder creeks etc.) install barriers at the mouth of these landscape features as well.

The invasion of Ontario waterways by *S. aloides* presents a difficult challenge for researchers, managers, governments, and residents. The solutions to this problem are complex and will continue to evolve over time. In the meantime, the species continues to thrive and expand its spatial scale. It is however, crucial to continue efforts to manage *S. aloides* to establish control in its current locations and effectively prevent further colonization in the Trent-Severn Waterway and beyond.

BIBLIOGRAPHY

Abu Ghalwa, N., Abu-Shawish, H.M., Hamada, M., Hartani, K., Basheer, A. A. H. 2012. Studies on Degradation of Diquat Pesticide in Aqueous Solutions Using Electrochemical Method. American Journal of Analytical Chemistry 3:99-105.

Alberta Agriculture and Forestry. 2016. Herbicide Group Classification by Mode of Action. Available at: http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/prm6487.

Anton-Hough, R., Fornwall, M., Negele, B., Thompson, R., Putt, D. 1989. Plant Community Dynamics in a Chain of Lakes: Principal Factors in the Decline of Rooted Macrophytes with Eutrophication. Hydrobiologia 173: 199-217.

Aquatic Plant Management Society, Inc. (APMS). 2016. A Manager's Definition of Aquatic Plant Control. Accessed May 2016 from: http://apms.org/resources/control/.

Ayala, R., Acosta, F., Mooij, W., Rejas, D., Van Damme, P. 2007. Management of Laguna Alalay: A Case Study of Lake Restoration in Andean Valleys in Bolivia. Aquatic Ecology 41:621-630.

Bal, K., Meire, P. 2009. The Influence of Macrophyte Cutting on the Hydraulic Resistance of Lowland Rivers. Journal of Aquatic Plant Management 47: 65-68.

Bakker, E., Van Donk, E., Immers, A. 2016. Lake Restoration by In-Lake Iron Addition: A Synopsis of Iron Impact on Aquatic Organisms and Shallow Lake Ecosystems. Aquatic Ecology 50: 121-135.

Barko, J., Gunnison, D., Carpenter, S. 1991. Sediment Interactions with Submersed Macrophyte Growth and Community Dynamics. Aquatic Botany 41: 41-65.

Barko, J., Hardin, D., Matthews, M. 1982. Growth and Morphology of Submersed Freshwater Macrophytes in Relation to Light and Temperature. Canadian Journal of Botany 60: 877-887.

Barko, J., Smart, R. 1981. Comparative Influences of Light and Temperature on the Growth and Metabolism of Selected Submersed Freshwater Macrophytes. Ecological Monographs 51: 219-236.

Barko, J., Smart, R. 1986. Sediment-Related Mechanisms of Growth Limitation in Submersed Macrophytes. Ecology 67: 1328-1340.

Basiouny, F., Haller, W., Gerrard, L. 1978. Survival of Hydrilla (*Hydrilla verticillata*) Plants and Propagules After Removal from the Aquatic Habitat. Weed Science 26: 502-504.

Bellaud, M. 2014. Cultural and Physical Control of Aquatic Weeds. In: Biology and Control of Aquatic Plants. Eds. Gettys, L., Haller, W., Petty, D. Aquatic Ecosystem Restoration Fund. Marietta, GA.

Best, E., Visser, H. 1987. Seasonal Growth of the Submersed Macrophyte *Ceratophyllum demersum L*. in Mesotrophic Lake Vechten in Relation to Insolation, Temperature and Reserve Carbohydrates. Hydrobiologia 148: 231-243.

Bergeron, C., Minteer C., Evans, C., Moorhead, D., Douce, K., Reardon, R. 2008. Invasive Plants of the United States. Accessed April 2016 from: http://www.invasive.org/weedcd/.

Bickel, T., Closs, G. 2009. Impact of Partial Removal of the Invasive Macrophyte *Lagarosiphon major* (Hydrocharitaeae) on Invertebrates and Fish. River Research and Applications 25: 734-744.

Binzer, T., Sand-Jensen, K. 2002a. Importance of Structure and Density of Macroalgae Communities (*Fucus serratus*) for Photosynthetic Production and Light Utilization. Marine Ecology Progress Series 235: 53-62.

Binzer, T., Sand-Jensen, K. 2002b. Production in Aquatic Macrophyte Communities: A theoretical and Empirical Study of the Influence of Spatial Light Distribution. Limnology and Oceanography 47:1742-1750.

Binzer, T., Sand-Jensen, K., Middelboe, A. 2006. Community Photosynthesis of Aquatic Macrophytes. Limnology and Oceanography 51:2722-2733

Bodkin, P., Posluszny, U., Dale, H. 1980. Light and Pressure in Two Freshwater Lakes and Their Influence on the Growth, Morphology and Depth Limits of *Hippuris vulgaris*. Freshwater Biology 10: 545-552.

Boeger, R. 1992. The Influence of Substratum and Water Velocity on Growth of *Ranunculus aquatilis* L. (Ranunculaceae). Aquatic Botany 42: 351-359

Bonan, G. 2002. Ecological Climatology: Concepts and Applications. Cambridge University Press, Cambridge, UK. Accessed online from: http://www.cgd.ucar.edu/tss/aboutus/staff/bonan/ecoclim/1sted/Chapter09.pdf.

Bornette, G., Puijalon, S. 2011. Response of Aquatic Plants to Abiotic Factors: A Review. Aquat Sci 73; 1-14.

Bowes, G., Van, T., Garrard, L., Haller, W. 1977. Adaption to Low Light Levels By Hydrilla. Journal of Aquatic Plant Management 15: 32-35.

Bowmer, K. 1982a. Adsorption Characteristics of Seston in Irrigation Water: Implications for the use of Aquatic Herbicides. Australian Journal of Marine and Freshwater Research 33:443-458.

Bowmer, K. 1982b. Aggregates of Particulate Matter and Aufwuchs on *Elodea canadensis* in Irrigation Waters, and Inactivation of Diquat. Australian Journal of Marine and Freshwater Research 33: 589-593.

Brammer, E., 1979. Exclusion of Phytoplankton in the Proximity of Dominant Water-soldier (*Stratiotes aloides*). Freshwater Biology 9: 233-249.

Bultemeier, B., Netherland, M., Ferrell, J., Haller, W. 2009. Differential Herbicide Response Among Three Phenotypes of *Cabomba caroliniana*. Invasive Plant Science and Management 2: 352-359.

Caffrey, A., Hoyer, M., Canfield Jr, D. 2007. Factors Affecting the Maximum Depth of Colonization by Submersed Macrophytes in Florida Lakes. Lake and Reservoir Management 23:287-297

Campbell, K., Bartell, S., Chaw, J. 2000. Characterizing Aquatic Ecological Risks From Pesticides Using a Diquat Dibromide Case Study. II. Approaches Using Quotients and Distributions. Environmental Toxicology and Chemistry 19: 760-774

Canfield, D., Langeland, K., Linda, S., Haller, W. 1985. Relations Between Water Transparency and Maximum Depth of Macrophyte Colonization in Lakes. Journal of Aquatic Plant Management 23: 25-28.

Canning, R. 2012. The Biology and Management of *Stratiotes aloides*: Honours Thesis Field Measurements. Unpublished. Trent University, Ontario.

Capers, R., Selsky, R., Bugbee, G., White, J. 2005. A Guide to Invasive Aquatic Plants of Connecticut. The Connecticut Agricultural Experiment Station. Accessed from: http://www.staffordvillelake.com/Forms/Aquatics%20Guide.pdf.

Caraco, N., Cole, J., Findlay, S., Wigand, C. 2006. Vascular Plants as Engineers of Oxygen in Aquatic Systems. BioScience 56: 219-225.

Carpenter, S., Lodge, D. 1986. Effects of Submerged Macrophytes on Ecosystem Processes. Aquatic Botany 26: 341-370.

Chambers, P., Kalff, J. 1985. Depth Distribution and Biomass of Submersed Aquatic Macrophyte Communities in Relation to Secchi Depth. Canadian Journal of Fisheries and Aquatic Science 42: 701-709.

Clark, D.G., Hurst, E.W. 1970. The Toxicity of Diquat. British J Industr Med 27;51-55.

Clayton, J., Matheson, F. 2010. Optimizing Diquat Use for Submerged Aquatic Weed Management. Hydrobiology 656: 159-165.

Clements, D., Dugdale, T., Hunt, T. 2013. Determining the Efficacy of the Herbicides Endothal and Diquat on the Aquatic Weed Sagittaria in Irrigation Channels. Plant Protection Quarterly 28: 85-87

Colautti, R., Bailey, S., van Overdijk, C., Amundsen, K., MacIsaac, H. 2006. Characterized and Projected Costs of Nonindigenous Species in Canada. Biological Invasions 8:45-59.

Cook, C., Urmi-Konig, K. 1983. A Revision of the genus *Stratiotes* (*Hydrocharitaceae*). Aquatic Botany 16: 213-249.

Cuda, J. 2009. Introduction to Biological Control of Aquatic Weeds. In: Biology and Control of Aquatic Plants. Eds. Gettys, L., Haller, W., Petty, D. Aquatic Ecosystem Restoration Fund. Marietta, GA.

Dale, M. 1986. Temperature and Light: The Determining Factors in Maximum Depth Distribution of Aquatic Macrophytes in Ontario, Canada. Hydrobiologia 133: 73-77.

Dar, N.A., Pandit, A.K., Ganai, B.A. 2014. Factors Affecting the Distribution Patterns of Aquatic Macrophytes. Limnol Rev 14;2;75-81.

Dawson, F., Robinson, W. 1984. Submersed Macrophytes and the Hydraulic Roughness of a Lowland Chalkstream. Verhandlungen des Internationalen Verein Limnologie 22: 1944-1948.

De Geus-Kruyt, M., Segal, S. 1973. Notes on Productivity of *Stratiotes aloides* in Two Lakes in the Netherlands. Polish Archives of Hydrobiology 20: 195-205.

Dennison, W. 1987. Effects of Light on Seagrass Photosynthesis, Growth and Depth Distribution. Aquatic Botany 27: 15-26.

Dennison, W., Orth, R., Moore, K., Stevenson, C., Carter, V., Kollar, S., Bergstrom, P., Batiuk, R. 1993. Assessing Water Quality with Submersed Aquatic Vegetation. BioScience 43: 86-94.

Dugdale, T., Clements, D., Hunt, T., Butler, K. 2012. Survival of a Submerged Aquatic Weed (*Egeria densa*) During Lake Drawdown within Mounds of Stranded Vegetation. Lake and Reservoir Management 28: 153-157.

Dukes, J., Mooney, H. 1999. Does Global Change Increase the Success of Biological Invaders? Tree 14: 135-139.

Efremov, A., Filonenko, A., Sviridenko, B. 2015. Anatomy and Morphology of Reproductive Organs of *S. aloides L.* (Hydrochariaceae). Inland Water Biology 8:4:334-344.

Efremov, A., Sviridenko, B. 2008. The Ecobiomorph of Water Soldier *Stratiotes aloides L.* (Hydrocharitaceae) in the West Siberian Part of Its Range. Inland Water Biology, 1;3;225-230.

Efremov, A., Sviridenko, B. 2012. Seasonal and Spatial Dynamics of *Stratiotes aloides* (Hydrocharitaceae) Plants. Botanica Serbica 36: 59-62.

Erixon, G. 1979. Population Ecology of a *Stratiotes aloides L*. Stand in a Riverside Lagoon in N. Sweden. Hydrobiologia 67: 215-221.

Ferrell, J. 2014. Spray Adjuvants: A User's Guide. In: Biology and Control of Aquatic Plants. Eds. Gettys, L., Haller, W., Petty, D. Aquatic Ecosystem Restoration Fund. Marietta, GA.

Figueroa, F., Salles, S., Aguilera, J., Jimenez, C., Mercado, J., Vinegla, B., Flores-Moya, A., Altamirano, M. 1997. Effects of Solar Radiation on Photoinhibition and Pigmentation in the Red Alga *Porphyra leucostica*. Marine Ecology Progress Series 151: 81-90

Forbes, R. 2000. Assessing the Status of *Stratiotes aloides* L. (Water-soldier) in Co. Fermanagh, Northern Ireland. Watsonia 23: 179-196.

Fox, A., Haller, W., Getsinger, K. 1991. Herbicide Application Technique Development for Flowing Water. Summary of research accomplishments. U.S. Army Engineer Waterways Experiment Station. Vicksburg, MS.

Fox, A., Murphy, K. 1990. The Efficacy and Ecological Impacts of Herbicide and Cutting Regimes on the Submerged Plant Communities of Four British Rivers. Journal of Applied Ecology 27: 520-540.

Franklin, P., Dunbar, M., Whitehead, P. 2008. Flow Controls on Lowland River Macrophytes: A Review. Science of the Total Environment 400: 369-378.

Funderburk, H., Lawrence, J. 1964. Mode of Action and Metabolism of Diquat and Paraquat. Weeds 12: 259-264

Getsinger, K., Fox, A., Haller, W. 1996. Herbicide Application Technique Development for Flowing Water: Summary of Research Accomplishments. U.S Army Corps of Engineers. Washington, DC.

Getsinger, K., Green, W., Westerdahl, H. 1990. Characterization of Water Movement in Submersed Plant Stands. US Army Engineer Waterways Experiment Station. Vicksburg, MS.

Getsinger, K., Poovey, A., Glomski, L., Slade, J., Richardson, R. 2011. Utilization of Herbicide Concentration/Exposure Time Relationships for Controlling Submersed Invasive Plants on Lake Gaston, Virginia/North Carolina. U.S. Army Corps of Engineers. Washington, DC.

Getsinger, K., Westerdahl, H. 1986. Evaluation of 2,4-D/ Adjuvant Mixtures in Flowing Water. U.S Army Engineer Waterways Experiment Station. Vicksburg, MS.

Ginn, B. 2011. Distribution and Limnological Drivers of Submerged Aquatic Plant Communities in Lake Simcoe (Ontario, Canada): Utility of Macrophytes as Bioindicators of Lake Trophic Status. Journal of Great Lakes Research 37:83-89.

Glime, J. 2007. Bryophyte Ecology: Volume 1. Michigan Technological University. Accessed from: http://www.bryoecol.mtu.edu/.

Glomski, L., Netherland, M. 2007. Efficacy of Diquat and Carfentrazone-ethyl on Variable-leaf Milfoil. Journal of Aquatic Plant Management 45:136-138.

Glomski, L., Skogerboe, J., Getsinger, K. 2005. Comparative Efficacy of Diquat to Two Members of the Hydrodcharitaceae Family: *Elodea and hydrilla*. Journal of Aquatic Plant Management 43:103-105.

Haller,W. 2014. Mechanical Control of Aquatic Weeds. In: Biology and Control of Aquatic Plants. Eds. Gettys, L., Haller, W., Petty, D. Aquatic Ecosystem Restoration Fund. Marietta, GA.

Hanelt, D. 1992. Photoinhibition of Photosynthesis in Marine Macrophytes of the South China Sea. Marine Ecology Progress Series 82: 199-206

Harpenslager, S., Smolders, A., Kieskamp, A., Roelofs, J., Lamers, L. 2015. To Float or Not to Float: How Interactions between Light and Dissolved Inorganic Carbon Species Determine the Buoyancy of Stratiotes aloides. PLoS One 10: 1-17

Haslam, S. 1978. River Plants: The Macrophytic Vegetation of Water-courses. Cambridge University Press, London, UK.

Havel, J., Kovalenko, K., Thomas, S., Amalfitano, S., Kats, L. 2015. Aquatic Invasive Species: Challenges for the Future. Hydrobiologia 750: 147-170.

Health Canada. 2009. The Regulation of Pesticides in Canada. Accessed May 2016 from: http://www.hc-sc.gc.ca/cps-spc/pubs/pest/_fact-fiche/reg-pesticide/index-eng.php.

Helton, R., Hartmann, L. 1995. Control of Waterhyacinth by Winter Drawdown. Proceedings of the Annual Conference of the Southeast Association of Fish and Wildlife Agencies 49: 105-111

Herron, H., Mauzerall, D. 1970. Light Saturation Curve of Photosynthesis. Biochemical et Biophysica Acta Bioenergetics 205: 312-314

Hess, D.F. 2000. Light-dependent Herbicides: An Overview. Weed Science 48; 160-170.

Hobbs, R. 2007. Setting Effective and Realistic Restoration Goals: Key Directions for Research. Restoration Ecology 15: 354-357.

Hofstra, D., Clayton, J., Getsinger, K. 2001. Evaluation of Selected Herbicides for the Control of Exotic Submerged Weeds in New Zealand: II. The Effects of Turbidity on Diquat and Endothall Efficacy. Journal of Aquatic Plant Management 39: 25-27.

Hofstra, D., Clayton, J., Green, J., Auger, M. 1999. Competitive Performance of *Hydrilla Verticillata* in New Zealand. Aquatic Botany 63: 305-324.

Horsch, E., Lewis, D. 2009. The Effects of Aquatic Invasive Species on Property Values: Evidence from a Quasi-Experiment. Land Economics 85: 391-409.

Hudon, C., Lalonde, S., Gagnon, P. 2000. Ranking the Effects of its Exposure, Plant Growth Form, Water Depth, and Transparency on Aquatic Plant Biomass. Canadian Journal of Fisheries and Aquatic Science 57: 31-42.

Huppertz, K., Hanelt, D., Nultsch, W. 1990. Photoinhibition of photosynthesis in the Marine Brown Alga *Fucus serrates* as Studied in Field Experiments. Marine Ecology Progress Series 66: 175-182

Hussner, A., Hoelken, H., Jahns, P. 2010. Low Light Acclimated Submerged Freshwater Plants Show a Pronounced Sensitivity to Increasing Irradiances. Aquatic Botany 93: 17-24.

Hutchinson, E. 1975. A Treatise on Limnology. Volume III- Limnological Botany. John Wiley & Sons New York, NY.

Hyldgaard, B., Sorrell, B., Brix, H. 2014. Closely Related Freshwater Macrophyte Species, *Ceratophyllum demersum* and *C.submersum*, Differ in Temperature Response. Freshwater Biology 59: 777-788

IFAS, 2016. University of Florida Centre for Aquatic and Invasive Plants: Plant Management in Florida. Accessed May 2016 from: https://plants.ifas.ufl.edu/manage/control-methods/chemical-control/a-brief-history-of-aquatic-herbicide-use/.

Ismail, A., Ella, E., Vergara, G., Mackill, D. 2009. Mechanisms associated with tolerance to flooding during germination and early seedling growth in rice (*Oryza saliva*). Annals of Botany 103: 197-209.

Jimenez, C., Figueroa, F., Salles, S., Hader, D. 1998. Effects of Solar Radiation on Photosynthesis and Photoinhibition in Red Macrophytes from an Intertidal System of Southern Spain. Botanical Marina 49:321-338

Johnston, D., Sutton, D., Vandiver, V., Langeland, K. 1983. Replacement of Hydrilla by Other Aquatic Plants in a Pond with Emphasis on Growth of American Lotus. Journal of Aquatic Plant Management 21:41-43.

Jordan, L., Murashige, T., Mann, J., Day, B. 1966. Effect of Photosynthesis-Inhibiting Herbicides on Non-Photosynthetic Tobacco Callus Tissue. Weeds 14: 134-136

Kaenel, B., Uehlinger, U. 1999. Aquatic Plant Management: Ecological Effects in Two Streams of the Swiss Plateau. Hydrobiologia 415: 257–263

Kenworthy, W., Fonseca, M. 1996. Light: Requirements of Seagrasses *Halodule wrightii* and *Syringodium filforme* Derived from the Relationship Between Diffuse Light Attenuation and Maximum Depth of Distribution. Estuaries 19: 740-750.

Klein, J., Verlaque, M. 2011. Experimental Removal of the Invasive Caulerpa Racemosa Triggers Partial Assemblage Recovery. Journal of the Marine Biological Association of the United Kingdom 91: 117-125.

Kornatowski, J. 1979. Turions and Offsets of *Stratiotes aloides L*. Acta Hydrobiologica Sinica. 21: 185-204.

Kornatowski, J. 1983. Morphological Forms of the Water soldier (*Stratiotes aloides L.).*

Acta Hydrobiologica Sinica. 25: 145-156.

Koyama, K., Kikuzawa, K. 2009. Is Whole-Plant Photosynthetic Rate Proportional to Leaf Area? A Test of Scalings and a Logistic Equation by Leaf Demography Census. The American Naturalist 173: 640-649.

Kufel, L., Strzalek, M., Konieczna, A., Izdebska, K. 2010. The effect of *Stratiotes aloides L.* and Nutrients on the Growth Rate of *Lemna minor L.* Aquatic Botany 92: 168-172.

Lacoul, P., Freedman, B. 2006. Environmental Influences on Aquatic Plants in Freshwater Ecosystems. Environmental Reviews. 14: 89-136.

Langeland, K., Sutton, D. 1980. Regrowth of Hydrilla from Auxillary Buds. Journal of Aquatic Plant Management 18: 27-29.

Lambers, H., Chapin III, F., Pons, T. 2008. Plant Physiological Ecology. Springer Science and Business Media LLC. New York, NY

Linhart, J. 1999. Phytophilous Macrofauna in the *Stratiotes Aloides* Vegetation of the Lake Lukie, Poland. Biologica 37: 76-76.

Locke, A., Mandrake, N., Therriault, T. 2010. A Canadian Rapid Response Framework for Aquatic Invasive Species. DFO Canada Science Advisory Secretariat Research Document 2010/114. vi+30p.

Lodge, D. 1991. Herbivory on Freshwater Macrophytes. Aquatic Botany 41: 195-224.

Lougheed, V., Crosby, B., Chow-Fraser, P. 2001. Primary Determinants of Macrophyte Community Structure in 62 Marshes Across the Great Lakes Basin: Latitude, Land Use, and Water Quality Effects. Canadian Journal of Fisheries and Aquatic Science 58:1603-1612.

Lovell, S.J., Stone, S.F. 2005. The Economic Impacts of Aquatic Invasive Species: A Review of the Literature. U.S. Environmental Protection Agency, National Center for Environmental Economics, Washington, D.C., USA.

MacDonald, F. 2009. Ontario Invading Species Awareness Program: Water Soldier. Acessed September 2016 from: http://www.invadingspecies.com/invaders/plants-aquatic/water-soldier/

Mache, A., Loiseaux, S. 1973. Light Saturation of Growth and Photosynthesis of the Shade Plant *Marchantia Polymorpha*. Journal of Cell Science 12: 391-401

Mack, R., Lonsdale, W. 2002. Eradicating Invasive Plants: Hard-won Lessons for Islands. In: Turning the tide: the eradication of invasive species. IUCN SSC Invasive Species Specialist Group. IUCN, Gland, Switzerland and Cambridge, UK.

Madsen, J. 2014. Developing a Lake Management Plan. In: Biology and Control of Aquatic Plants. Eds. Gettys, L., Haller, W., Petty, D. Aquatic Ecosystem Restoration Fund. Marietta, GA.

Madsen, J., Chambers, P., James, W., Koch, E., Westlake, D. 2001. The Interaction Between Water Movement, Sediment Dynamics and Submersed Macrophytes. Hydrobiologia 444: 71-84.

Madsen, J., Hartleb, C., Boylen, C. 1991. Photosynthetic Characteristics of *Myriophyllum* spicatum and Six Submersed Aquatic Macrophyte Species Native to Lake George, New York. Freshwater Biology 26: 233-240

Madsen, T., Sand-Jensen, K. 1994. The Interactive Effects of Light and Inorganic Carbon on Aquatic Plant Growth. Plant, Cell and Environment 17: 955-962.

Madsen, T., Warncke, E. 1983. Velocities of Currents Around and Within Submerged Aquatic Vegetation. Archives Hydrobiologia. 97:389.

Marshall, D. 1986. Effect of Seed Size on Seedling Success in Three Species of Sesbania (*Fabaceae*). American Journal of Botany 73: 457-464.

Marshall, E., Westlake, D. 1990. Water Velocities Around Water Plants in Chalk Streams. Folia Geobotanica et Phytotaxonomica 25: 279-289.

Masclef, A., 1891. Stratiotes aloides L. Accessed November 2016 from: https://upload.wikimedia.org/wikipedia/commons/a/a2/348_Stratiotes_aloides_L.jpg. Used with permission from Wikimedia Commons

McAllister, L. 1991. Factors Influencing the Distribution of Submerged Macrophytes in Green Bay, Lake Michigan: A Focus on Light Attenuation and *Vallisneria americana*. Environmental Science and Policy Master's Thesis. University of Wisconsin-Green Bay.

McCreary, N. 1991. Competition as a Mechanism of Submersed Macrophyte Community Structure. Aquatic Botany 41: 177-193.

McGehee, J. 1979. Mechanical Hydrilla Control in Orange Lake, Florida. Journal of Aquatic Plant Management 17: 58-61.

McKnight, K., Hepp, G. 1995. Potential Effect of Grass Carp Herbivory on Waterfowl Foods. Journal of Wildlife Management 59: 720-727.

Mebane, C., Simon, N., Maret, T. 2013. Linking Nutrient Enrichment and Streamflow to Macrophytes in Agricultural Streams. Hydrobiologia 722: 143-158.

Mees, G. 1960. Experiments on the Herbicidal Action of 1,1'-Ethylene-2,2'-Dipyridylium Dibromide. Annals of Applied Biology 3:601-612

Meinesz, A. 1999. Killer Algae: The True Tale of a Biological Invasion. University of Chicago Press, Chicago, IL.

Michigan Department of Agriculture and Rural Development, (MDARD). 2015. Weed Risk Assessment for *Stratiotes aloides* L. (Hydrocharitaceae) - Water soldier. Pesticide and Plant Pest Management Division. Lansing, Michigan.

Middleboe, A.L., Markager, S. 1997. Depth Limits and Minimum Light Requirements of Freshwater Macrophytes. Freshwater Biology, 37;553-568.

Millenium Ecosystem Assessment. 2005. Ecosystems and Human Well Being: Synthesis. Island Press, Washington, D.C., USA.

Ministry of Environment (MOE). 2009. Ministry of the Environment Ontario Pesticide Training Core Manual. Queens Printer, Toronto ON.

Moss, B., Jeppesen, E., Sondergaard, M., Lauridsen, T., Liu, Z. 2013. Nitrogen, Macrophytes, Shallow Lakes and Nutrient Limitation: Resolution of a Current Controversy? Hydrobiologia 710: 3-21.

Mulderij, G., Mau, B., de Senerpont Domis, L., Smolders, A., Van Donk, E. 2009. Interaction Between the Macrophyte *Stratiotes aloides* and Filamentous Algae: Does it Indicate Allelopathy? Aquatic Ecology 43: 305-312.

Mulderij, G., Mooij, W., Smolders, A., Van Donk, E. 2005. Allelopathic Inhibition of Phytoplankton by Exudates from *Stratiotes aloides*. Aquatic Botany 82: 284-296.

Myers, J., Kitajima, K. 2007. Carbohydrate Storage Enhances Seedling Shade and Stress in a Neotropical Forest. Journal of Ecology 95: 383-395.

Myers, J., Simberloff, D., Kuris, A., Carey, J. 2000. Eradication Revisited: Dealing with Exotic Species. Tree 15:316-320.

Necchi, O. 2004. Light Related Photosynthetic Characteristics of Lotic Macroalgae. Hydrobiologia 525: 139-155

Netherland, M. 2014. Chemical Control of Aquatic Weeds. In: Biology and Control of Aquatic Plants. Eds. Gettys, L., Haller, W., Petty, D. Aquatic Ecosystem Restoration Fund. Marietta, GA.

Netherland, M. 2013. Response of Water Soldier to the Herbicide Diquat: The Effect of Turbidity, Water Temperature and Plant Size on Efficacy. Final report to OMNRF Peterborough, ON.

Netherland, M., Schardt, J. D. 2014. A Manager's Definition of Aquatic Plant Control. In: Getty, L.A., Haller, W.T., Petty, D.G. Eds. Biology and Control of Aquatic Plants. A Best Management Practices Handbook: Third Edition. Aquatic Ecosystem Restoration Foundation, Marietta, Georgia.

Netherland, M.D., Skogerboe, J.D., Owens, C.S., Madsen, J.D. 2000. Influence of Water Temperature on the Efficacy of Diquat and Endothall Versus Curlyleaf Pondweed. J Aquat Plant Mgmt 38; 25-32.

Newman, R.M. 1991. Herbivory and Detritivory on Freshwater Macrophytes by Invertebrates: A Review. J North Am Benthol Society 10:2; 89-114.

Nielsen, S., Sand-Jensen, K. 1989. Regulation of Photosynthetic Rates of Submerged Rooted Macrophytes. Oecologia 81: 364-368

Nilsson, C., Gardfjell, M., Grelsson, G. 1991. Importance of Hydrochory in Structuring Plant Communities Along Rivers. Canadian Journal of Botany 69: 2631-2633.

Ogdahl, M., Steinmann, A. 2014. Factors Influencing Macrophyte Growth and Recovery Following Shoreline Restoration Activity. Aquatic Botany 120:363-370.

Olesen, B., Enriquez, S., Duarte, C., Sand-Jensen, K. 2002. Depth-acclimation of Photosynthesis, Morphology and Demography of *Posidonia oceanica* and *Cymodocea nodosa* in the Spanish Mediterranean Sea. Marine Ecology Progress Series 236: 89-97

Ontario Federation of Anglers and Hunters (OFAH). 2016. Trent River Water soldier Monitoring Program: Lake Seymour, Crowe Bay and Percy Reach. Peterborough, ON

Ontario Invasive Species Strategic Plan (OISSP). 2012. Ontario Invasive Species Strategic Plan: Economic Impacts. Queen’s Printer, Toronto, ON

Ontario Ministry of Agriculture and Rural Affairs (OMAFRA). 2016. Publication 75: Guide to Weed Control 2016-2017. Accessed May 2016 from: http://www.omafra.gov.on.ca/english/crops/pub75/pub75toc.htm.

Ontario Ministry of Natural Resources and Forestry (OMNRF). 2013. Ontario’s Invasive Aquatic Plant List. Accessed August 2014 from: https://www.ontario.ca/environment-and-energy/invasive-aquatic-plant-list.

Ontario Ministry of Natural Resources and Forestry (OMNRF). 2013. An Integrated Management Plan for Water Soldier (*Stratiotes Aloides L.)* in Ontario, Canada. DRAFT.

Ontario Ministry of Natural Resources and Forestry (OMNRF). 2015. Ontario Invading Species Awareness Program 2015 Water soldier Distribution Map. Accessed September 2016 from: http://www.invadingspecies.com/invaders/plants-aquatic/water-soldier/

Opioła, J. 2009. Stratiotes aloides. Accessed November 2016 from: https://upload.wikimedia.org/wikipedia/commons/thumb/a/a8/Stratiotes_aloides_1-OB9.JPG/128px-Stratiotes_aloides_1-OB9.JPG. Used with permission under GNU Free Documentation License and Wikimedia Commons

Otahelova, H., Banasova, V. 1997. Population Structure and Reproduction of *Stratiotes aloides* in the Morava River Floodplain. Biologia Bratislava 52: 523-529.

Owens, C., Madsen, J. 1995. Low Temperature Limits of Water Hyacinth. Journal of Aquatic Plant Management 33: 63-68.

Panel on the Future of the Trent-Severn Waterway, 2008. Report of the
The Panel on the Future of the Trent-Severn Waterway. Accessed May April 2016 from: http://www.tswpanel.ca/english/report.asp.

Parks Canada. 2016. Trent Severn Waterway: National Historic Site. Accessed November 2016 from: http://www.pc.gc.ca/eng/lhn-nhs/on/trentsevern/index.aspx

Pedersen, O., Colmer, T., Sand-Jensen, K. 2013. Underwater Photosynthesis of Submerged Plants- Recent Advances and Methods. Frontiers in Plant Science 4:1-19

Pieczyriska, E., Ozimek, T., Rybak, J. 1988. Long-term Changes in Littoral Habitats and Communities in Lake Mikoajskie (Poland). International Review of Hydrobiology 73: 361-378.

Pilon, J., Santamaría, L. 2002. Clonal Variation in Morphological and Physiological Responses to Irradiance and Photoperiod for the Aquatic Angiosperm *Potamogeton pectinatus*. Journal of Ecology 90: 859-870

Pimentel, D., Lach, L., Zuniga, R., Morrison, D. 2000. Environmental and Economic Costs of Nonindigenous Species in the United States. BioScience 50: 53-65.

Poovey, A., Getsinger, K. 2002. Impacts of Inorganic Turbidity on Diquat Efficacy Against *Egeria densa*. Journal of Aquatic Plant Management 40:6-10.

Poovey, A., Getsinger,K., Skogerboe, J., Koschnick, T., Madsen, J., Stewart, R. 2004. Small-Plot, Low-Dose Treatments of Triclopyr for Selective Control of Eurasian Watermilfoil. Lake and Reservoir Management 20: 322-332.

Rantala, M., Ilmonen, J., Koskimaki, J., Shone, J., Tynkkynen, K. 2004. The Macrophyte, Stratiotes aloides, Protects Larvae of Dragonfly Aeshna viridis Against Fish Predation. Aquatic Ecology 38: 77–82

Rasmussen, J. 2000. Mississippi Interstate Cooperative Resource Association: Summary of Permit Authority and Prohibited Species by State With Special Emphasis on Asian Carp. Accessed March 2014 from: http://www.asiancarp.org/Regulations/ansregus%20MRB.pdf.

Rahel, F., Bierwagen, B., Taniguchi, Y. 2008. Managing Aquatic Species of Conservation Concern in the Face of Climate Change and Invasive Species. Conservation Biology 22: 551-561.

Renman, G. 1989a. Life Histories of Two Clonal Populations of *Stratiotes aloides L.* Hydrobiologia 185: 211-222.

Renman, G. 1989b. Distribution of Littoral Macrophytes in a North Swedish Riverside Lagoon in Relation to Bottom Freezing. Aquatic Botany 33: 243-256.

Roelofs, J. 1991. Inlet of Alkaline River Water into Peaty Lowlands: Effects on Water Quality and Stratiotes Aloides L. Stands. Aquatic Botany 39: 267-293

Richards, A. 1986. Plant Breeding Systems Second Edition. Chapman and Hall London, UK.

Richardson, R. 2008. Aquatic Plant Management and the Impact of Emerging Herbicide Resistance Issues. Weed Technology 22: 8-15.

Riis, T., Biggs, B. 2001. Distribution of Macrophytes in New Zealand Streams and Lakes in Relation to Disturbance Frequency and Resource Supply: A Synthesis and Conceptual Model. New Zealand Journal of Marine and Freshwater Research 35: 255-267.

Ritter, A., Shaw, J., Martin Williams, W., Travis, K. 2000. Characterizing Aquatic Ecological Risks From Pesticides Using a Diquat Dibromide Case Study. I. Probabilistic Exposure Estimates. Env Tox and Chem 19;3; 749-759.

Sand-Jensen, K. 1989. Environmental Variables and Their Effect on Photosynthesis of Aquatic Plant Communities. Aquatic Botany, 34; 5-25.

Sand-Jensen, K. 1997. Broad-Scale Comparison of Photosynthesis in Terrestrial and Aquatic Plant Communities. Oikos 80: 203-208

Sand-Jensen, K., Binzer, T., Middelboe, A. 2007. Scaling of Photosynthetic Production of Aquatic Macrophytes. Oikos 116: 280-294

Sand-Jensen, K., Madsen, T. 1991. Minimum Light Requirements of Submerged Freshwater Macrophytes in Laboratory Growth Experiments. Journal of Ecology 79: 749-764.

Sand-Jensen, K., Pederson, O. 1999. Velocity Gradients and Turbulence Around Macrophyte Stands in Streams. Freshwater Biology 42: 315-328

Sand-Jensen, K., Sondergaard, M. 1981. Phytoplankton and Epiphyte Development and Their Shading Effect on Submerged Macrophytes in Lakes of Different Nutrient Status. Internationale Revue der gesamten hydrobiology und Hydrographie 66: 529-552.

Salvucci, M., Bowes, G. 1981. Induction of Reduced Photorespiratory Activity in Submersed and Amphibious Aquatic Macrophytes. Plant Physiology 67: 335-340

Santamaría, L., van Vierssen, W. 1997. Photosynthetic Temperature Responses of Fresh and Brackish Water Macrophytes: A Review. Aquatic Botany 58: 135-150

Sarneel, J. 2013. The Dispersal Capacity of Vegetative Propagules of Riparian Fen Species. Hydrobiologia Online 201210.1007/s10750-012- 1022-3.

Schutten, J., Davy, A. 2000. Predicting the Hydraulic Forces on Submerged Macrophytes From Current Velocity, Biomass and Morphology. Oecologia 123:445-452.

Schwartz, A., Howard-Williams, C., Clayton, J. 2000. Analysis of Relationships Between Maximum Depth Limits of Aquatic Plants and Underwater Light in 63 New Zealand Lakes. New Zealand Journal of Marine and Freshwater Research 34: 157-174.

Sculthorpe, C. 1967. The Biology of Aquatic Vascular Plants. Edward Arnold, London, England.

Siemering, G.S., Hayworth, J.D. 2008. Assessment of Potential Aquatic Herbicide Impacts to California Aquatic Ecosystems. Arch Environ Contam Toxicol 55:415-431.

Simberloff, D. 2003. How Much Information on Population Biology is Needed to Manage Introduced Species? Conservation Biology 17:83-92.

Simberloff, D., Parker, I., Windle, P. 2005. Introduced Species Policy, Management, and Future Research Needs. Frontiers in Ecology and the Environment 3: 12-20.

Simsiman, G.V., Chesters, G. 1976. Persistence of Diquat in the Aquatic Environment. Water Research 10:105-112.

Skogerboe, J., Getsinger, K., Glomski, L. 2006. Efficacy of Diquat on Submersed Plants Treated Under Simulated Flowing Water Conditions. Journal of Aquatic Plant Management 44: 122-125.

Smolders, A., den Hartog, C., Roelofs, J. 1995a. Observations on Fruiting and Seed-Set of *Stratiotes aloides* L. in the Netherlands. Aquatic Botany 51: 259-268.

Smolders, A., den Hartog, C., Roelofs, J. 1995b. Germination and Seedling Development in *Stratiotes aloides* L. Aquatic Botany 51: 269-279.

Smolders, A., den Hartog, C., van Gestal, C., Roelofs, J. 1996b. The Effects of Ammonium on Growth, Accumulation of Free Amino Acids and Nutritional Status of Young Phosphorous Diffident *Stratiotes aloides* Plants. Aquatic Botany 53: 85-96

Smolders, A., Lamers, L., den Hartog, C., Roelofs, J. 2003. Mechanisms Involved in the Decline of *Stratiotes aloides L*. in The Netherlands: Sulphate as a Key Variable. Hydrobiologia 506: 603-610.

Smolders, A., Roelofs, J., den Hartog, C. 1996c. Possible Causes for the Decline of the Water soldier (*Stratiotes aloides* L.) in the Netherlands. Acta Hydrobiologica Sinica 136: 327-342

Smolders, A., Roelofs, J. 1995. Internal Eutrophication, Iron Limitation and Sulphide Accumulation Due to the Inlet of River Rhine Water in Peaty Shallow Waters in the Netherlands. Acta Hydrobiologica Sinica 133: 349-365

Smolders, A., Roelofs, J. 1996. The Roles of Internal Iron Hydroxide Precipitation, Sulphide Toxicity and Oxidizing ability in the Survival of *Stratiotes aloides* Roots at Different Iron Concentrations in Sediment Pore Water. New Phytologist 133: 253-260

Smolders, A., Roelofs, J., den Hartog, C. 1996a. Possible Causes for the Decline of the Water soldier (*Stratiotes alludes L.)* in the Netherlands. Archiv ruer Hydrobiologie 136: 327-342.

Sondergaard, M. 1988. Photosynthesis of Aquatic Plants Under Natural Conditions pp 63-111. In eds. Symoens, J. 1988. Vegetation of Inland Waters. Kluwer Academic Publishers. Dordrecht, NL

Spence, D. 1975. Light and Plant Response in Fresh Water. In: Light as an Ecological Factor: II. Eds., Evans, G., Bainbridge, R., Rackham, O. Blackwell Scientific Productions, Oxford.

Spence, D., Chrystal, J. 1970. Photosynthesis and Zonation of Freshwater Macrophytes. New Phytologist 69:205-215

Spencer, D., Anderson, L., Ames, M., Frederick, R. 1987. Variation in *Hydrilla verticillata (L.f.*) Royle Propagule Weight. Journal of Aquatic Plant Management 25: 11-14.

Spencer, D., Ksander, G. 2001. Comparison of Light Compensation Points for Two Submersed Macrophytes. Journal of Freshwater Ecology 16: 509-515

Strzałek, M., Koperski, P. 2009. The *Stratiotes aloides L*. Stand as a Habitat in Oxbow Lake Buzysko. Aquatic Botany 90: 1-6.

Sugier, P., Lorens, B., Chmiel, S., Turcztnski, M. 2010. The influence of *Ceratophyllum demersum L.* and *Stratiotes aloides L.* on richness and diversity of aquatic vegetation in the lakes of mid-eastern Poland. Hydrobiologia 656: 43-53.

Suhonen, J., Scutari, E., Kaunisto, K., Krams, I. 2013. Patch Area of Macrophyte Stratiotes aloides as a Critical Resource for Declining Dragonfly Aeshna viridis. Journal of Insect Conservation 17: 393-398

Suutari, E., Salmela, J., Paasivirta, L., Rantala, M., Tynkkynen, K., Loujumaki, M., Suhonen, J. 2009. Macroarthopod Species Richness and Conservation Priorities in *Stratiotes aloides* (L.) Lakes. Journal of Insect Conservation 13: 413-419.

Syngenta Canada Inc. 2015. Reward: Aquatic Herbicide Product Label. Guelph, ON

Syngenta Crop Protection LLC. 2014. Reward: Landscape and Aquatic Herbicide Label. Greensboro, NC

Tanaka, N., Uehara, K., Murata, J. 2004. Correlation Between Pollen Morphology and Pollination Mechanisms in the Hydrocharitaceae. Journal of Plant Research 117: 265-276.

Tanner, C., Clayton, J., Coffey, B. 1990. Submerged-Vegetation changes in Lake Rotoroa (Hamilton, New Zealand) Related to Herbicide Treatment and Invasion by Egeria Densa. New Zealand Journal of Marine and Freshwater Research 24: 45-58

Tanner, C., Wells, R., Mitchell, C. 1990a. Re-establishment of Native Macrophytes in Lake Parkinson Following Weed Control by Grass Carp.
New Zealand Journal of Marine and Freshwater Research 24: 181-186

Titus, J., Adams, M. 1979a. Coexistence and the Comparative Light Relations of the Submersed Macrophytes Myriophyllum spicatum L. and Vallisinseria americana Michx. Oecologia 40: 273-286

Titus, J., Adams, M. 1979b. Comparative Carbohydrate Storage and Utilization Patterns in the Submersed Macrophytes, *Myriophyllum spicatum* and *Vallisneria americana*. American Midland Naturalist 102: 263-272.

Toma, C. 2006. Distribution and Comparison of Two Morphological Forms of Water Soldier (*Stratiotes aloides L.):* A Case Study on Lake Slosineckie Wielkie (Northwest Poland). Biodiversity Research and Conservation 4:251-257.

Toma, C. 2012. Reproduction of *Stratiotes aloides* in Ilmoilanselka Lake (Finland). Journal of Ecology and Protection of the Coastline 16: 103-111.

Tor Nielson, L., Borum, J. 2008. Why the Free Floating Macrophyte *Stratiotes aloides* Mainly Grows in Highly CO2-Supersaturated Waters. Aquatic botany 89: 379-384.

Torn, K., Martin, G., Kotta, J., Krupp, M. 2010. Effects of Different Types of Mechanical Disturbances on a Charophyte Dominated Macrophyte Community. Estuarine, Coastal and Shelf Science 87:27-32.

Unmuht, J., Sloey, D., Lillie, R.1998. An Evaluation of Close-cut Mechanical Harvesting of Eurasian Watermilfoil. Journal of Aquatic Plant Management 36: 93–100

USDA. 2014. United States Department of Agriculture PLANTS Database: *Stratiotes L.* Accessed February 2014 from: http://plants.usda.gov/core/profile?symbol=STRAT.

Van, T., Haller, W., Bowes, G. 1976. Comparison of the Photosynthetic Characteristics of Three Submersed Aquatic Plants. Plant Physiology 58: 761-768.

Van den Berg, M., Joosse, W., Coops, H. 2003. A Statistical Model Predicting the Occurrence and Dynamics of Submerged Macrophytes in Shallow Lakes in the Netherlands. Hydrobiologia 506-509: 611-623.

van der Bijl, L., Sand-Jensen, K., Hjermind, A. 1989. Photosynthesis and Canopy Structure of a Submerged Plant, Potamogeton Pectinatus, in a Danish Lowland Stream. Journal of Ecology 77:947-962

Van der Winden, J., Beinteam, A., Heemskerk, L. 2004. Habitat-related Black Tern (Chlidonias niger) Breeding Success in The Netherlands. Ardea 92: 53–61

Van Donk, E., De Deckere, E., Klein Breteler, J., Meulemans, J. 1994. Herbivory by Waterfowl and Fish on Macrophytes in a Biomanipulated Lake: Effects on Long-term Recovery. Verhandlungen des Internationalen Verein Limnologie 25: 2139-2143.

van Oosterhout, E., Johnson, A., Ensbey, R. 2013. Department of Primary Industries Weed Alert: Water soldier. Assessed August 2014 from: http://www.dpi.nsw.gov.au/agriculture/pests-weeds/weeds/profiles/water-soldier.

Veen, C., Sarneel, J., Ravensbergen, L., Huig, N., Van Passen, J., Rip, W., Bakker, E. 2013. Aquatic Grazers Reduce the Establishment and Growth of Riparian Plants Along an Environmental Gradient. Freshwater Biology 58: 1794-1803

Wang, C., Zheng, S., Wang, P., Qian, J. 2014. Effects of Vegetations on the Removal of Contaminants in Aquatic Environments: A Review. J Hydrodynamics 26:4; 497-511.

Washington, T., Sidnei, T. 2003. Effects of Light on the Growth and Photosynthesis of *Egeria rajas Planchon*. Brazilian Archives of Biology and Technology 46: 203-209.

Weissflog, N., Sager, E. 2016. An Investigation of the Reproductive Ecology of Crab's-claw in the Trent River, Ontario, Canada. Journal of Aquatic Plant Management 54:72-77

Wes, M., 1980. The Effects of Propagule Size on the Germination and Seedling Growth in *Mirabilis hirsuta*. Canadian Journal of Botany 60: 1868-1874.

Wetzel, R., Likens, G. 1991. Limnological Analysis. Springer-Verlag Publishing New York, NY.

White, A., Reiskind, J. 1996. Dissolved Inorganic Carbon Influences the Photosynthetic Response of Hydrilla to Photoinhibitory Conditions. Aquatic Botany 53: 3-13.

Wilcox, D., Whillans, T. 1999. Technique for the Restoration of Disturbed Coastal Wetlands of the Great Lakes. Wetlands 19: 835-857.

Williams, E.H., Mather, E.L., Carter, S.M. 1984. Toxicity of the Herbicides Endothall and Diquat to Benthic Crustacea. Bull Environ Contam Toxicol 33:418-422.

Witt, A. 2003. Low Photoinhibition Among Submerged Macrophytes. In Environmental Future of Aquatic Ecosystems, 5th International Conference on Environmental Future. March 23-27, 2003

Wolverton, B., McDonald, R. 1979. Water Hyacinth (Eichhornia Crassipes) Productivity and Harvesting Studies. Economic Botany 33: 1-10

Xu, W., Hu, W., Deng, J., Zhu, J., Li, Q. 2014. Effects of Harvest Management of *Trapa bispinosa on* an Aquatic Macrophyte Community and Water Quality in a Eutrophic Lake. Ecological Engineering 64: 120-129.

Yeo, R. 1967. Dissipation of Diquat and Paraquat and Effects on Aquatic Weeds and Fish. Weeds 15: 42-46.

Zimmerman, R., Cabello-Pasini, A., Alberte, R. 1994. Modeling Daily Production of Aquatic Macrophytes from Irradiance Measurements: A Comparative Analysis. Marine Ecology Progress Series 114: 185-196.

APPENDIX 1: Diquat Label (Canada)

GROUP 22 HERBICIDE

REWARD®

Aquatic Herbicide

Solution

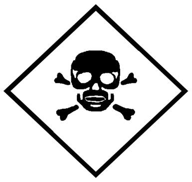
RESTRICTED

For control of weeds in still or slow-moving water of farm dugouts, farm ponds, industrial ponds, farm ditches, lakes, streams and canals, and for weed control in non-crop land (rights-of-way for transportation or utility corridors, airports, wasteland, garbage dumps and industrial parks).

GUARANTEE:
Diquat ion .... 240 g per litre
(present as dibromide)

READ THE LABEL AND BOOKLET BEFORE USING
KEEP OUT OF REACH OF CHILDREN

WARNING



POISON

CAUTION – EYE AND SKIN IRRITANT

REGISTRATION NO. **26271**
PEST CONTROL PRODUCTS ACT

Syngenta Canada Inc.
140 Research Lane, Research Park
Guelph, Ontario
N1G 4Z3
Telephone: 1-877-964-3682

WARNING!
***HARMFUL OR FATAL IF SWALLOWED.**
HARMFUL IF INHALED, AVOID INHALING/BREATHING DUST, SPRAYS, ETC.
***CAUSES SUBSTANTIAL EYE INJURY AND SKIN IRRITATION.**
***DO NOT GET IN EYES, ON SKIN OR ON CLOTHING.**
***NEVER TRANSFER TO OTHER CONTAINERS.**
*** KEEP OUT OF REACH OF CHILDREN AND ANIMALS.**

NOTICE TO USER

This pest control product is to be used only in accordance with the directions on the label. It is an offence under the *Pest Control Products Act* to use this product in a way that is inconsistent with the directions on the label. The user assumes the risk to persons or property that arises from any such use of this product.

NATURE OF RESTRICTION: This product is to be used only in the manner authorized, consult local pesticide regulatory authorities about use permits which may be required.

The restricted uses of REWARD® Aquatic Herbicide may be subject to other legislative requirements such as those under the Fisheries Act.

RESTRICTED USES

For the control of water weeds such as Coontail **(*Ceratophyllum* spp.)**, Duckweed **(*Lemna* spp.)**, Canada Water Weed **(*Elodea, Anacharis* spp.)**, Pondweeds **(*Potamogeton* spp. except Richardson's Pondweed (*P.richardsonni*))**, Water Milfoil **(*Myriophyllum* spp.),** Water Soldier **(*Stratiotes aloides*)**, Water Chestnut **(*Trapa natans* L.)**, and Flowering Rush **(*Butomus umbellatus*)** in still or slow flowing water of farm dugouts, farm ponds, industrial ponds, farm ditches, lakes, streams and canals, apply REWARD® Aquatic Herbicide at 18.3 L/ha (1 ha = 10,000 m^2 or 100 m x 100 m). 2.3 litres of REWARD Aquatic Herbicide will treat a farm dugout 25 m x 50 m. Milfoil may be controlled at 9.2 L/ha in the early stages of growth. For the control of growing weeds in 1.5 m of water or less, use 18.3 L/ha of REWARD Aquatic Herbicide. For the control of growing weeds in more than 1.5 m of water, apply 25-29.2 L/ha of REWARD Aquatic Herbicide.

Algae: *Cladophora, Spirogyra*, and *Pithophora* spp. will be temporarily controlled at the above rates. Repeat treatments may be required for full season control of these species. REWARD Aquatic Herbicide will not control Stonewort, Muskgrass (***Chara* spp.**). REWARD Aquatic Herbicide, after suitable dilution with clean water, may be injected below the water surface, sprayed over the water surface or poured directly onto the surface while moving over the surface in a boat, or may be applied from the banks of small bodies of water.

To inject below the water surface, a suction type of boat bailer is mounted on the cavitation plate of an outboard motor and the end of the inlet tube inserted into a solution containing 1 part REWARD Aquatic Herbicide diluted with at least 10 parts of clean water. Make lines of travel at regular intervals through the water (3 m or less apart) over the area to be treated until the whole area has received a uniform application.

For Surface application, dilute 1 part REWARD Aquatic Herbicide with at least 4 parts clean water and spray over water surface, pour directly onto water surface or apply from the banks of small bodies of water.

Timing of Application: Apply only after weeds are visible and in an active stage of growth which is normally sometime in late May through June as growth is dependent on water temperatures. Application should be made to actively growing weeds before they become so thick that they make application difficult. **Application to dense growth of mature weeds will not give satisfactory control.**
Repeat treatments may be required if weed growth reappears. Repeat application of diquat to water bodies is prohibited for two weeks after application in order to protect aquatic organisms. For temporary control of water weeds growing above the surface of the water, such as waterlilies, uniformly spray with REWARD Aquatic Herbicide at 9.2 to 26.7 L in 1700 to 2200 L of water per hectare to thoroughly wet the foliage. Use AGRAL® 90 at 1 L per 1000 L of water.

LIMITATIONS

In some provinces of Canada permits are required before chemicals such as REWARD Aquatic Herbicide can be added to water. Consult local authorities before applying REWARD Aquatic Herbicide to ascertain whether such a permit is required in your area. Do not use treated water for at least 24 hours after treatment for swimming and animal consumption. For human consumption and irrigation do not use for at least 5 days after treatment. To protect the fish in small lakes, streams and ponds with a dense weed growth, treat not more than 1/4 to 1/3 of the area at one time, otherwise the dying weeds over a large area will cause a serious loss of oxygen which may injure or kill the fish. Do not apply to muddy water and do not agitate water excessively during 1 or 2 days after treatment or the effectiveness of the chemical will be reduced. Use clean water for diluting the chemical.

Do not use wetting agents for water treatment, except as specified. Avoid application or drift onto crops, ornamental plants, lawns, grazing areas or other desirable growth. Do not apply through mist blowers.

It is important to thoroughly wash equipment after spraying - use a detergent or wetting agent (AGRAL 90 at 60 mL per 100 L of water), flush and spray out, then thoroughly rinse with clean water. When possible, the equipment should be filled with clean water and left overnight. Spray out before storing equipment or using for other materials.

FIRST AID

Take container, label or product name and Pest Control Product Registration Number with you when seeking medical attention.

If swallowed, call a poison control centre or doctor **IMMEDIATELY** for treatment advice. Have person sip a glass of water if able to swallow. Do not induce vomiting unless told to do so by a poison control centre or doctor. Do not give anything by mouth to an unconscious person.

If in eyes, IMMEDIATELY hold eye open and rinse slowly and gently with water for 15–20 minutes. Remove contact lenses, if present, after the first 5 minutes, then continue rinsing eye. Call a poison control centre or doctor for treatment advice.

If on skin or clothing, take off contaminated clothing. Rinse skin **IMMEDIATELY** with plenty of water for 15–20 minutes. Call a poison control centre or doctor for treatment advice.

APPENDIX 2: Dissolved Oxygen Measurements from the July 25th and August 29th Incubation Arrays

July 25th	Dissolved Oxygen Measurements (µmol)								
Depth (m)	**Bottle**	**Array 1_Initial**	**Array 1_Final**	**Array 2_Initial**	**Array 2_Final**	**Array 3_Initial**	**Array 3_Final**	**Array 4_Initial**	**Array 4_Final**
0	L1	7.42	15.49	7.33	12.23	7.86	11.9	7.79	14.81
	L2	7.558	14.2	7.37	16.5	7.71	13.56	7.79	14.56
	D	7.44	6.06	7.33	6.95	7.74	4.58	7.84	5.77
	B	7.46	7.73	7.48	7.58	7.66	7.53	7.61	7.66
1	L1	7.19	11.92	7.07	17.22	7.9	14.34	7.77	15.26
	L2	7.48	15.89	7.64	13.64	7.79	13.34	7.6	17.18
	D	7.6	5.38	7.1	7.07	7.72	5.1	7.63	5.89
	B	7.39	7.8	7.74	7.9	7.7	7.88	7.75	8.06
2	L1	7.14	11.35	7.56	14.48	7.53	14.3	7.74	16.28
	L2	7.11	7.8	7.69	12.69	7.54	14.77	7.86	13.98
	D	7.18	5.81	7.32	5.47	7.59	5.55	7.72	4.48
	B	7.53	7.84	7.69	7.96	7.76	7.9	7.81	8.15
3	L1	7.28	10.43	7.45	10.08	7.58	11.64	7.56	10.16
	L2	7.19	10.57	7.84	14.07	7.73	12.3	7.54	8.89
	D	7.24	5.74	7.46	6.64	7.88	5.29	7.9	6.06
	B	7.43	7.7	7.62	8.07	7.45	7.82	7.73	8.06
4	L1	7.29	6.46	7.59	9.99	7.92	9.07	7.74	7.58
	L2	7.49	8.11	7.79	9.14	7.7	8.93	7.77	7.8
	D	7.3	6.19	7.78	6.46	7.63	6.21	7.89	4.85
	B	7.43	7.67	7.71	7.7	7.73	7.71	7.89	7.91
5	L1	7.38	7.18	7.76	6.99	7.73	8.91	7.87	7.31
	L2	7.42	8.07	7.74	7.97	7.76	7.3	7.59	7.26
	D	7.23	6.24	7.52	5.98	7.79	6.11	7.61	4.14
	B	7.3	7.73	7.9	7.85	7.76	7.75	7.67	7.95
6	L1	7.45	7.03	7.77	6.71	7.62	7.04	7.61	6.77
	L2	7.3	7.12	7.7	6.88	7.52	5.57	7.83	6.26
	D	7.47	4.34	7.85	5.1	7.79	4.29	7.79	4.3
	B	7.22	7.66	7.65	7.84	7.7	7.89	7.8	8

August 29th	**Dissolved Oxygen Measurements (µmol)**								
Depth (m)	**Bottle**	**Array 1_Initial**	**Array 1_Final**	**Array 2_Initial**	**Array 2_Final**	**Array 3_Initial**	**Array 3_Final**	**Array 4_Initial**	**Array 4_Final**
0	L1	7.91	11.56	7.87	10.27	8.08	9.63	7.9	12.13
	L2	7.78	12.79	7.85	11.86	7.99	11.52	8.02	13.38
	D	7.86	7.7	8.04	6.35	9.98	6.74	7.87	6.38
	B	7.82	7.59	8.04	7.97	8.12	8.01	7.82	7.67
1	L1	7.8	10.74	8.24	10.72	7.95	11.28	7.93	12.1
	L2	7.89	12.22	7.92	11.17	7.94	10.87	7.98	14.22
	D	7.86	6.61	8.13	6.95	9.98	6.74	7.97	6.89
	B	7.94	8.11	7.95	8.12	8.12	8.01	7.9	8.15
2	L1	7.91	11.42			7.85	11.84	7.73	14.02
	L2	7.88	10.66			8.05	11.1	7.84	14.64
	D	7.91	6.78			8.03	6.78	7.97	6.82
	B	7.85	8.21			7.99	8.03	7.77	8.16
3	L1	8.09	10.21	8.08	10.2	7.93	10.92	7.84	11.75
	L2	8.09	8.1	8.2	11.01	7.93	11.15	7.96	8.6
	D	8.09	7.22	8.09	7.5	8.06	6.41	7.96	6.33
	B	8.13	8.45	8.3	8.33	8.05	8.2	7.77	7.88
4	L1	8.04	9.06	8.12	7.8	8.03	8.14	7.91	9.83
	L2	8.05	8.01	8.07	8.89	7.94	10.11	7.87	9.33
	D	8.07	6.82	8.06	7.28	7.95	7.4	8.01	6.45
	B	8.04	8.2	7.81	8.26	7.93	8.21	8.03	8.05
5	L1	7.95	8.3	8.31	8.17	7.73	8.9	8.02	7.76
	L2	8.06	8.08	8.14	8.43	7.87	8.08	8.05	7.95
	D	8.09	7.11	8.15	7.12	7.94	7.59	8.06	6.66
	B	7.89	8.36	8.11	8.04	7.81	8.08	8.02	8.12
6	L1	7.81	7.84	8.03	7.76	7.92	8	8.04	8.19
	L2	7.93	7.61	7.87	7.98	7.99	8.07	8.14	8.19
	D	7.89	7.59	8.05	7.22	7.98	8.02	8.11	6.78
	B	7.92	8.67	8.03	8.19	7.98	8.1	8.36	8.46

APPENDIX 3: Summary of Statistical Procedures and Results from Chapter 3

Parameter	Parametric Assumptions	Treatment Site Sample Period	Statistical Test	Post Hoc P Values
S. aloides Biomass	Pass (log transformed)	September	P=0.563; df=3; F value=0.7 (ANOVA)	N/A
		June	P=0.66; df=3; F value=0.541 (ANOVA)	N/A
		August	P=0.034; df=3; F value=1.926 (ANOVA)	Sphys=0.02; Schem=0.0004; Fphys=0.05 (Tukey HSD)
		October	P=0.00197; df=3; F value=7.131 (ANOVA)	Schem=0.00004; Sphys=0.01; Fphys=0.001 (Tukey HSD)
Local Biomass	Pass	September	P=0.0052; df=4; F value=4.785 (ANOVA)	Fphys=0.01; Fchem=0.02 (Tukey HSD)
		June	P=0.0146; df=4; F value=3.832 (ANOVA)	Fchem=0.01; Sphys=0.02 (Tukey HSD)
		August	P=0.419; df=4; F value=0.62 (ANOVA)	N/A
		October	P=0.003; df=4; F	Sphys=0.005;

			value=4.343 (ANOVA)	Fchem=0.001 (Tukey HSD)
Species Richness	Fail	September	P=0.06; df=4 (Kruskal-Wallis)	N/A
		June	P=0.33; df=4 (Kruskal-Wallis)	N/A
		August	P=0.58; df=4 (Kruskal-Wallis)	N/A
		October	P=0.001; df=4 (Kruskal-Wallis)	Fchem=0.001 (Pairwise Tukey Nemenyi)
Shannon-Wiener Index	Fail	September	P=0.85; df=4 (Kruskal-Wallis)	N/A
		June	P=0.73; df=4 (Kruskal-Wallis)	N/A
		August	P=0.009; df=4 (Kruskal-Wallis)	Fchem=0.01; Fphys=0.01 (Pairwise Tukey Nemenyi)
		October	P=0.001; df=4 (Kruskal-Wallis)	Fchem=0.003 (Pairwise Tukey Nemenyi)