Wild rice (*Zizania palustris L.*) Re-establishment Through Mechanical Removal of Invasive Cattails (*Typha angustifolia* L.)

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Abstract

Re-establishment of wild rice following the removal of the invasive cattail, *Typha angustifolia*, was examined in a traditional and culturally important stand of wild rice belonging to the Seine River First Nation on Rainy Lake, Ontario. The site selected for study had no wild rice production for over twenty-five years. The objectives of the study were i) to evaluate the effectiveness of mechanical removal of cattails as a method for restoring wild rice habitat; ii) to examine the impact of invasive cattails on long-term nutrient dynamics in the wild rice stand: and iii) to determine if the presence of cattails impacted the germination of wild rice. It was hypothesized that the removal of the cattails would permit wild rice re-establishment.

An underwater sickle bar attached to an airboat was used to cut and remove cattails in a treatment area in Rat River Bay in the fall of 2014. This field experiment showed no cattail regrowth in the spring of 2015. A second result was the germination of wild rice in the cut area from the seed bank which grew to maturity by the fall of 2015.

Plant tissue, porewater and sediment macro and micronutrients were analyzed from a cattail site, a natural wild rice site not impacted by cattails and the wild rice site created from the cut cattail area. Results showed that plant tissue concentrations in the natural wild rice site were significantly higher than the cut site for potassium, carbon and nitrogen content. On a per unit area basis, cattails had significantly higher concentrations in their tissue for nitrogen phosphorus, potassium, carbon, calcium, magnesium and sulfur. These resulted in significantly lower plant tissue concentrations per unit area for nitrogen, phosphorus and potassium in the cut wild rice site versus the natural wild rice site. Similar trends occurred in the porewater and sediment for

major macronutrients. Chlorosis was evident in the cut area likely due to nitrogen deficiency Cattails had significantly lower nitrogen concentrations in porewater and sediment and significantly lower phosphorus concentrations in the sediment. Discriminant analysis using porewater and sediment variables was able to separate the three treatment sites. In 2015, the natural wild rice site was classified with 95% accuracy, the treated site with 86.7% accuracy and the cattail site with 58% accuracy, and most commonly misclassified as the treated site. By 2017, the cattail site was classified separately from the other sites with an 83.3% accuracy while wild rice and the treated site were less distinct and most often misclassified as one or the other sites.

The potential impact of cattails on the germination of wild rice was examined using a mesocosm and controlled experiments in a growth chamber. The mesocosm consisted of six 42 l buckets with cattails and six without cattails. Each bucket had a monitoring well that was sampled weekly for two months for pH, dissolved oxygen, temperature, conductivity and redox. Water was continually flowing into the mesocosm. Germination experiments were conducted with wild rice seeds in petri plates and 200 ml beakers placed in a growth chamber with a 16hr, 25°C, 8hr 12°C day/night cycle. Experiments consisted of a.) cattail litter from roots, rhizomes and leaves plus wild rice seed, and b.) distilled water plus wild rice seed, distilled water plus wild rice seed and aerated, and distilled water plus wild rice seed bubbled with nitrogen gas to achieve an anaerobic environment. Results from the mesocosm demonstrated that cattails in the buckets lowered pH and increased oxidation reduction potential versus buckets with no cattails. The germination experiments revealed that wild rice germination was significantly higher in the cattail rhizome and root treatments versus the cattail leaves. Germination was also significantly higher in the anaerobic treatment versus the other treatments.

The findings of the study showed that successful re-establishment of wild rice could occur if the cattails were removed with negligible cattail regrowth. Cattails did have an impact on the nutrient environment causing depletion of macro and micronutrients with nitrogen depletion being particularly noticeable. This was thought to have a long-term impact on the sustainability of the re-established wild rice. Germination of wild rice was stimulated by conditions causing a more reduced environment. The suggested mechanism for the loss of germination in wild rice was the release of oxygen from cattail rhizomes which resulted in a loss of wild rice germination. Our hypothesis that removal of cattails could result in wild rice re-establishment was correct but longer-term studies are needed to determine if the wild rice would produce sustainable populations given the alteration of the sediment by cattails.

Overall, the research provided new insights into how invasive cattails can alter the environment to eliminate a native species. The research also demonstrated that these effects may be reversed by removing the cattails and permit the re-establishment of traditional wild stands in Northwestern Ontario.

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This thesis is dedicated to my partner Matt and my family, without your wholehearted support and free labour, I could not have reached this goal.

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Literature Review

Wetlands - Value and Impact of Invasion

Wetlands are simply defined by the presence of water, unique soil conditions and vegetation that is adapted to flooded conditions (Mitsch and Gosselink 2000). Wetlands cover 6% of the surface of the Earth's, yet they contribute 40% of the global annual ecosystem services (Costanza 1997, Mitsch and Gosselink 2000). These services including food production, water regulation and purification, as well as retention and transformation of chemical and biological materials (Costanza et al.1998, Woodward and Wui 2001, Pagiola et al. 2004). Wetlands also provide habitat for a considerable number of aquatic plants and animals including a high percentage of endangered and threatened species (Flather et al. 1998, Mitsch and Gosselink 2000). Costanza et al. (1997) valued the global annual ecosystem services of wetlands at $14,785/ha per year compared to temperate/boreal forest valued at $302/ha per year. However, since 1900 an estimated 64-71% of wetlands have been classified as highly degraded to completely destroyed (Davidson 2014) putting additional stress on existing wetlands and an emphasis on the restoration of degraded wetlands.

Wetlands degradation often occurs through plant invasions in which they are extremely vulnerable (Zedler and Kercher 2004). Invasive species are able to establish, proliferate and persist in a new environment. Their continuous spatial and temporal expansion displaces native species and is a leading cause of biodiversity loss (Wilcove et al. 1998, Richardson et al. 2000, Chaplin et al. 2000, Mack et al. 2000, Zedler and Kercher 2004, Mitsch and Gosselink 2007). The loss in diversity results in the degradation of wetland function and services (D'Antonio and Vitousek 1992, Wilcove at al 1998, Parker et al. 1999 Mack et al. 2000, Ehrenfeld 2003, Zedler

and Kercher 2005, Nentwig 2007) including carbon sequestering and retention (Farnsworth and Meyerson 2003, Smith et al. 2018), water cycling/purification, and nutrient retention (Vitousek 1986, Ehrenfeld 2003, Lee et al. 2017). Due to the increasing frequency and impact of invasion (di Castri 1989, Cohen and Carlton 1998, Mack et al 2000), there is an ever-increasing need for effective and sustainable restoration (Vitousek 1997, Mack et al. 2000, Levinel et al. 2003, Zedler and Kercher 2004, Tuchman 2009)

Wild Rice

Wild rice (*Zizania palustris* L.), is Canada's only native cereal crop (Archibold et al. 1985) and has significant cultural, economic and ecological value (Jenks 1899). The First nations people have harvested wild rice for centuries and it is an integral part their diet. Their settlement in Northwester Ontario, is believed to have coincided with wild rice (Moyle 1944, Dore 1969, Johnson 1969, Ackley 2000, Schlender 2000). There is also evidence that First Nation's communities have contributed to the wide distribution throughout lakes in Northwestern Ontario (Boyd et al. 2012). High nutritional values including various antioxidants and, low-fat content make wild rice a healthy natural food source (Surendiran et al. 2014). It has been cultivated commercially since 1930 and was once an important part of the economy of First Nations communities in northwestern Ontario (Lee and Stewart 1984). Presently, the demand for wild rice far exceeds current world production, and one acre of natural wild rice can produce up to 500 pounds of wild rice seed with a value of $ 0.75 to $13 per pound (MNDR 2008). Wild rice provides habitat as well as an important food source for mammals, fish, invertebrates (Moyle 1944), and a considerable portion of migratory birds (Dore 1969, McAtee 1917).

Wild rice forms dense continuous stands (Moyle 1944, Painchaud and Archibold 1990) and mature plants can reach a height of 2.5m (Grava and Raisanen 1978). They are protogynous with a single terminal panicle, female pistillate positioned above the male staminate. The staminate fall away from the panicle on flexible branches with the anthers exposed (Moyle 1944, Dore 1969). As an annual plant wild rice's life cycle starts again each year from seed. Germination occurs in the spring with the emergence of cotyledon, where the shoot is formed followed by a limited root system. The seed is resource limited, and the shoot must reach the surface of the water as quickly as possible (Chambliss 1940). Once this occurs, the floating leaf stage begins, and photosynthesis can begin. The floating leaf stage, lasts approximately 2 weeks and is followed by the formation of the emergent stem and leaves (Weir and Dale 1960). Next is the transition from vegetative to a reproductive shoot apex (Wier and Dale 1960), where tillering will occur under optimal conditions (Aikens et al. 1988). Once mature, the wind pollination blows the bearing sac into erect female spikelets of neighbouring plants. (Aiken et al. 1988). Shedding occurs four weeks after fertilization in late August to early September. The seed will fall from the parent plant with a rubber-like awning and unbalanced weight ensuring it doesn't travel far (Aiken et al. 1988, Lee 1979).

The induction of germination and viability during dormancy of wild rice seed is critical for the re-establishment of productive wild rice stands. Wild rice seed enters a primary dormancy during the afterrippening period that begins after separation from the parent plant. This afterrippening period occurs in the cold, anoxic sediment for a minimum of 3 months, ending in spring when conditions become more favourable for survival (Simpson 1966, Atkins 1986). Germination is believed to be cued from a combination of environmental factors such as temperature, oxygen

and light. Germination does not occur at a temperature below 4°C and optimal germination occurs at a fluctuating diurnal temperature (Simpson 1966, Atkins 1986). There has been conflicting evidence regarding the role of oxygen concentration in germination. Svare (1960) reported increased germination with decreased dissolved oxygen, but others reported high germination without deoxygenation (Simpson 1966, Campiranon and Koukkari 1977). Secondary dormancy occurs when primary dormancy is overcome but the viable seed does not germinate (Gutek 1975), but there is very little is known about what influences viability and secondary dormancy in wild rice.

Wild rice has a natural distribution in the southern boreal forest of central and eastern Canada (Painchaud and Archibold 1990). Water depth, climate, light penetration and sediment all factor into the success wild rice (Archibold et al. 1985). Wild rice is found exclusively in saturated sediment with overlying moving water but they are confined by a maximum depth of 1m. Water level depth is crucial to plant survival in all life stages (Moyle 1944, Dore 1969 Painchaud and Archibold 1990) and fluctuations of over 0.30m can potentially be fatal (Moyle 1944). Wild rice requires a minimal temperature range between 4 and 6°C for germination to occur and approximately 110 growing season days to reach maturity (Dore 1969). Wild rice requires a high level of light penetration, while deep waters, shading or turbulence can be detrimental (Dore 1969, Lee 1979). Northern wild rice prefers organic sediments but can survive in a wide range of sediment and water types (Aikens et al. 1988, Day and Lee 1989, Lee and McNaugthon 2004).

Wild rice does not compete well with other emergent macrophytes, and is rarely found in intermixed stands even under optimal growing conditions (Aiken et al. 1989, Dore 1969). As

Clay and Oelke (1987) have reported that wild rice yield can be reduced as much as 60% from the presences of giant bur-reed (*Sparganium eurycarpum* Engelm.). On Lake of the Woods, Gilbert (1985) showed that *Nuphar* spp were able to completely eradicated stands of wild rice, supported by the field experiments of Atkins (1983) that this would occur regardless of water depth. Wild rice has been reported to grow unimpeded with *Nymphaea ordorata* Aiton., *Ceratophyllum demersum* L., and *Vallisneria americana* Michx. (Atkins 1986). Although, there are no specific studies on the effects of cattails on wild rice. Research has shown the competitive exclusion of wild rice in high nutrient sites and that interspecific competition is detrimental to productivity of wild rice (Atkins 1984, Lee and Stewart 1984, Aiken et al 1989).

Cattails

Large natural stands of wild rice in the Rainy Lake watershed are being threatening by artificial water level management and the subsequent invasion of *T. angustifolia*. This non-native cattail, is a highly aggressive invasive macrophyte (Grace and Wetzel 1982, Mitchell et al. 2011), that thrives in disturbed ecosystem (Boers and Zedler 2008). Since 1938, the International Joint Commision (IJC) implemented control of water levels on Rainy Lake. An annual rule curve was implemented in 1949, which was revised in 2000, with the goal of narrowing the fluctuations of the upper and lower water level, to mimic natural water level fluctuations. Rat River Bay, a historically productive wetland had, no wild rice harvested in 2014 compared to historical commercial sales of up to 150,000 pounds (Lee 2015). These stands are of immense cultural, ecological and economic importance to the First Nations of this region and re-establishment of wild rice in the area is a priority.

Typha spp. are large emergent wetland macrophytes with approximately 30 species found worldwide (Apfelbaum 1985, Nowinska et al. 2014, Smith 1967). This genus experiences prolific growth in the early spring, followed by release of seeds in early fall, then the leaves senesce and the plant enters a dormant state for the winter (Sojda and Solberg 1993). The plant experiences dense clonal growth, producing several rhizomes that can spread laterally up to 70 cm (Hotchkiss and Dozier 1949, Smith 1967, Grace and Harrison 1986). The rhizomes provide a carbohydrate storage that allow the plant to spread quickly (Hotchkiss and Dozier 1949, Smith 1967, Linde et al. 1976, Grace and Harrison 1986). This genus produces 4-20 narrow linear basal leaves that taper off at the end (Morton 1975, Apfelbaum 1985), and are monoecious with the male staminate located above the pistillate portion in a spike shaped inflorescence (Linde et al. 1976).

Both of the common broadleaf cattail (*T. latifolia*) and invasive narrow leaved cattail (*T angustifolia*) are found in Northwestern Ontario. The invasive *T. angustifolia* is larger and more prolific, dominating a larger niche as they are less restricted by water depth and fluctuating water level (Harris and Marshall 1963). The invasive *T. angustifolia* is distinguishable from the native species by a 2-12 cm gap between the pistillate and staminate portion as well as its narrower leaves typically 5-11 cm wide (Hotchkiss and Dozier 1949, Linde et al. 1976,). *T. glauca* Godr. is a naturally occurring hybrid between *T. angustifolia* and *T. latifolia* with the ability to back cross which creates a high variability and can cause taxonomic difficulties. Although, *T. glauca* is infertile, it is more prolific and dominating than both its parent species (Waters and Shay 1990, Shih and Finkelstein 2008).

Initially, *T. angustifolia* was believed to have arrived on the Atlantic Coast with European settlement (Grace and Harrision, 1986, Woo and Zedler 2002), but recent studies using pollen records has shown establishment prior to settlement (Shih and Finkelstein, 2008). These findings suggest that *T. angustifolia* may be native to Northeastern United States, and that it advanced through the United States and into the Great Lakes region late in the 19th century (Hotchkiss and Dozier 1949, Shih and Finkelstein 2008). The records of The Claude Garton Herbarium at Lakehead University show this species was present in Northwestern Ontario in 1985. According to Seine River First Nations councillor, John Kabatay, and Chief Tom Johnson (personal communication), the plant was first noticed on their traditional lands in the late1980's to early 1990's but has only become prevalent in their wild rice stands in the last decade.

The invasive *T. angustifolia* has traits and regeneration strategies that make them successful. The high accumulation of nutrients in plant tissue due to large size and dense stands in addition to the slowly decay (Davis and Van der Valk 1983, Mack et al. 2000) reduces nutrient availability to native vegetation (Xiong and Nilson 1999, Vaccaro et al. 2009). The increased biomass accumulation as litter also plays an important role in preventing germination of native vegetation (Xiong and Nilson 1999) and release of chemical inhibitors (allelochemicals) during decomposition (McNaughton 1968, Jarchow and Cook 2009). *T. angustifolia* colonizes quickly by sexual reproduction with 20,000 to 70, 0000 fruits per inflorescence (Prunster 1941). Furthermore, *Typha* does not require a dormancy period and can retain 100% viable in the seed bank for up to 70 years, allowing germination to occur whenever conditions are favorable (Wienhold and Van Der Valk 1989, Sojda and Solberg 1993). The species also uses robust rhizome networks to reproduce vegetatively, that allows for early emergence and translocation of resources to ensure establishment (McNaughton 1966, Farnsworth and Meyerson 2003, Pysek

and Richardson 2007, Elgersma et al. 2015). *T. angustifolia* has a broad tolerance for environmental conditions including hydrology, nutrients, salinity, and heavy metal contamination which increases chances of naturalization (Farnworth and Meyerson 2003, Manios et al. 2003, Jacob and Otte 2004, Bailey-Serres and Voesenek 2008, Colmer and Voesenek 2009, Smith et al. 2015, Bonanno and Cirelli, 2017). Such traits and strategies create a positive feedback that diminishes the survival of native vegetation and contributes to the dominance of the invader (Mack et al. 2000, Tuchman et al. 2009).

Typha, like many emergent macrophytes, alleviates the stress of an anoxic environment through aerenchymous lacunae tissue. The tissues form a series of continuous open spaces that allow for oxygen transport from exposed leaves to submerged roots. This movement of oxygen meets the respiratory demands of submerged organs (Armstong 1972) and rhizosphere oxidation occurs as oxygen diffuses out the roots termed radial oxygen loss (Armstrong 1971). Creating an aerobic zone that prevents absorption of phytotoxins such as excessive iron, manganese and sulfide (Armstrong 1967, Mendelssohn and Postek 1982, Sorrell and Dromgoole 1987, Armstrong et al. 1992, Sorrell and Armstrong 1994). *T. angustifolia* increase oxygen transport efficiency by creating an internal pressurization and connective flow through (Brix et al. 1992, Tornbjerg et al. 1994) that allows them to grow in greater water depths (Tornbjerg et al. 1994) and increases the radial oxygen loss from the rhizosphere (Armstrong and Armstrong 1990).

The impacts of the hybrid invasive *T. glauca* on nutrients dynamics has been thoroughly examined (Tuchman et al. 2009, Larkin 2012, Geddes et al. 2014). It has a strong competition for nitrogen and is slow to decompose leaving fewer nutrients for native vegetation (Larkin 2012). The longer the invasion, the higher the concentration of soil organic matter, nitrate, ammonium

(Tuchman et al. 2009, Geddes et al. 2014) and phosphate (Angeloni et al. 2006) in sediment. Additionally, it was found that denitrification increases over time (Lishawa et al. 2014). Angeloni et al. (2006) discovered a change in bacterial and denitrifier communities in *T. glauca* invaded sites compared to noninvaded sites. The highest diversity of denitifier was found in sites invaded the longest, with a legacy effect seen as restored community remained similar to invaded community (Geddes et al. 2014). *T. glauca* thrives in high nutrient environments (Woo and Zedler 2002) which it often creates itself (Corbin and D'Antonio 2004, Angeloni et al. 2006, Jankowski 2007). This increase in nutrients disproportionately benefits the invader (Woo and Zedler 2002) and creates feedback that ensures dominance.

Typha Eradication

The biological effects and the loss of ecosystem services due to *Typha* invasion has led to the development of several control methods. These methods include chemical control with the use of glyphosate herbicide (Sojda and Solberg 1993, Linz et al. 2003, Solberg and Higgins 1993) However, herbicides must be adequately applied to kill cattails completely as any surviving cattails or rhizomes multiply rapidly. Although chemical control is successful and cost-effective, there are no herbicides registered in Canada for use on aquatic plants that compete with wild rice (Aiken et al.1988). Linde (1976) achieved the most success with shading in July when food resources of cattails are at their lowest. Unfortunately, it is not practical to cover acres of cattails, so this method is reserved for small areas. Burning methods can be successful if they occur while the water level is low, and then promptly raised to ensure the shoots are submerged (Ball 1990). The stocks must be submerged to kill the rhizomes and prevent vegetative

reproduction (Linde 1976, Sale and Wetzel 1983). However, this method is only suitable if control over water levels is possible.

The cutting and submergence of *Typha* is an effective method to reduce biomass (Nelson and Dietz 1966, Singh at al. 1973, Sale and Wetzel 1983, Jordan and Whigham 1988, Hellsten et al. 1999, Zedler, 2010, Lishawa at al. 2017). Studies show successful elimination after a single submerged cutting in stands of *T. angustrata* (Singh et al. 1973), *T. australis* (Hellsten et al. 1999), *T. angustifolia* (Husak 1978, Jordan and Whigham 1988), *T. latifolia* (Nelson and Dietz 1996) and *T. glauca* (Hall and Zedler 2010, Lishawa et al. 2015). Notably, some studies have reported regrowth after harvest (Buele 1979, Sharma and Kashwaha 1990, Kostecke et al. 2004, Tanaka et al. 2005 Lishawa et al. 2015), but differences in the success of harvesting illustrate the importance of submersion of cut stocks (Sale and Wetzel 1983, Hellsten et al. 1999, Tanaka et al. 2005). In contrast, Lishawa (2017) reported cutting without submergence was effective but only in young *Typha* stands. If cutting was repeated often enough that starch reserves were depleted to a point were vigorous regrowth was not possible (Hall and Zedler 2010, Lishawa et al. 2015, Linde et al. 1976).

The effectiveness of a control treatment will depend on the habitat, water depth and biology of the invader and native species (Corbin and D'Antonio 2012). Cutting and submergence of stocks is the most effective management option as it takes into consideration the target species morphology and physiology (Linde et al. 1976). Effective *Typha* eradication is dependent on the state of the underground stem as this perennial portion of the plant provides energy storage that is critical for survival during winter months and the production of new shoots for vegetative

reproduction (Linde et al. 1976). When a *Typha* stock is cut and submerged the oxygen supply is terminated (Breule 1979, Jordan and Whigman 1988, Hellsten et al. 1999) and conversion of starch to sugars ceases effectively severing their food source. This also induces anaerobic respiration and the subsequent production of ethanol which contributes to the rapid break down of plant tissue (Sale and Wetzel 1983), and therefore completely eliminating the threat of re-establishment (Lating 1941, Sojda and Solberg 1993 Hellsten et al. 1999). Studies also illustrate the importance of the timing of the cut, with a single submerged cut during flowering period when the rhizome's carbohydrate storage is at its lowest has shown to be most effective (Singh et al. 1973, Buele 1976, Hellsten 1999). The importance of submergence is magnified as Linde (1967) and Buele (1979) showed how damaged or injured rhizomes and stocks cause vigorous regrowth and proliferation of new shoots if not submerged.

Thesis Objectives

The objectives of the study were i) to evaluate the effectiveness of mechanical removal of cattails as a method for restoring wild rice habitat; ii) to examine the impact of invasive cattails on long-term nutrient dynamics in the wild rice stand: and iii) to determine if the presence of cattails impacted the germination of wild rice. It was hypothesized that the removal of the cattails would permit wild rice re-establishment.

Chapter 1

Re-establishment of Wild rice (*Zizania palustris* L.) from the seed bank following the removal of Invasive Cattails (*Typha angustifolia* L.)

1.1 Introduction

Adverse ecological impacts of plant invasion include habitat degradation, disturbance of trophic food webs, and the reduction of ecosystem goods and services (Vitousek et al. 1996, Wilcove et al. 1998, Ehrenfeld 2003, Zedler and Kercher 2004). Wetlands have a high incidence of disturbance due to their position in the landscape, as they are prone to excess water, sediment and nutrient loading (Green and Galatowitsch 2001, Woo and Zedler 2002). Invasions often precede such disturbances as invader traits are either better suited to the post-disturbance conditions and drive long term ecosystem change (Hobbs and Huenneke 1992, Burke and Grime et al. 1996, Ehrenfeld 2003, Levine et al. 2003). The invader-mediated changes in ecosystem condition may persist long after removal creating a legacy effect that influences restoration (Corbin and D'Antonio 2012). Legacy effects such as nutrient availability is often observed when invasive species eradicate native plants and alter nutrient resources by performing novel function (Suding et al. 2004, Corbin and D'Antonio 2012).

Invasive species often have higher growth rates and biomass production than native species resulting in changes in light availability, sedimentation, nutrient retention/availability and other biogeochemical processes (Whittaker 1965, Ehrenfeld 2003, Corbin and D'Antonio 2004, Currie 2014). Cattails (*T. glauca* and *T. angustifolia*) are common invasive species in wetlands, which can dramatically affect both southern wild rice (Lee 2005) and northern wild rice (Lee et al. 2016). The large size of *T. angustifolia* can cut down light to the neighbouring species by

shading the water column. The invasive *T. angustifolia* also experience low levels of herbivory leaving large amounts portions of biomass and the ground which plays a vital role in nutrient dynamics (Freyman 2008, Farrer and Goldberg 2009, Farrer 2014).

The impacts of the hybrid invasive *T. glauca* on nutrient dynamics is well documented (Geddes et al. 2014). Invasion duration increase the concentration of soil organic matter, nitrate, ammonium (Tuchman et al. 2009, Geddes et al. 2014) and phosphate (Angeloni et al. 2006) in sediment. Most importantly, *Typha* can impact nitrogen availability as slow decomposition results in net immobilization, which may coincide with the period of highest nutrient demand from neighbouring species (Berg and McClaugherty 2008). Davies et al. (1977) showed that *T. angustifolia* retains 50% of its total nitrogen content 525 days after senescence (Davis et al.1977). Additionally, it was found that denitrification was higher in sites invaded by cattails when compared to non-invaded sites (Geddes et al. 2014). It is well known that *T. glauca* thrives in high nutrient environments (Woo and Zedler 2002) which they often create themselves through nitrogen fixation and biomass accumulation (Corbin and D'Antonio 2004, Angeloni et al. 2006, Jankowski 2007). Such increases in nutrients disproportionately benefits the invader (Woo and Zedler 2002) and creates feedback that ensures dominance. This use of resources is perpetuated season after season, thus creating a novel ecosystem (Farnsworth and Meyerson 2003, Whittaker 1965) and making it distinctly different from typical wild rice stands (Lee and McNaugthon 2004, Ehrenfeld 2003, Callaway et al. 2004).

Zizania palustris L., northern wild rice is an annual aquatic grass and Canada's only naturally occurring cereal crop. This native plant has been a staple food for First Nations people for

centuries and has been cultivated commercially since the early 20th century. *Z. palustris* grows in shallow moving water in a range of sediment types. It is very sensitive to changes in water depth and turbidity and very rarely co-exists with other macrophytes due to its inability to compete (Clay and Oelke 1987, Gilbert 1985, Atkins 1983, Lee and Stewart 1984). Unlike perennials, wild rice is an annual plant, and it doesn't have the advantage of storing nutrients but must acquire all nutrients from the external environment each year (Day and Lee 1989, Painchaud and Archibold 1990). Therefore, it is more sensitive to resource deficiencies which are reflected in the oscillation of populations as well as plant size, colour and density (Sims et al. 2012, Keenen and Lee 1986). Nitrogen has been shown to be the most influential factor (Keenan and Lee 1986) limiting the growth and survivorship of wild rice (Sims et al. 2012). Hence, any changes to the natural cycling of nitrogen including legacy effects will impact the success of wild rice and needs to be a central factor during restoration (Lee 2017).

The most effective management plan of *Typha*, is mechanical harvesting (Nelson and Dietz 1966, Singh at al. 1973, Sale and Wetzel 1983, Jordan and Whigham 1988, Hellsten et al. 1999, Zedler 2010, Lishawa at al. 2017) but it is essential that cut stocks must remain submerged (Sale and Wetzel 1983, Hellsten et al. 1999, Tanaka et al. 2005). The disruption of oxygen is essential as it ensures the death of rhizome and decomposition. In addition, the cutting and removing of biomass have an increased potential for reducing legacy effects and recovering native diversity (Lishawa et al. 2015). However, its effectiveness is site-specific and restoration outcome will depend on site water depth and the creation of an alternative stable state (Sale and Wetzel 1983, Hellsten et al. 1999, Suding et al 2004, Tanaka et al. 2005).

Studies dealing with invasive plant species commonly explore their impact by investigating above ground processes such as changes in native plant diversity and productivity (Levine et al., 2003). Several studies have documented the relationship between the effects of invasive plants and nutrient cycling (Ehrenfeld 2003, Liao 2008). However, there are very few studies focusing on the impact of invader removal with the goal of native species restoration (Reynolds et al. 2017). Currently, there has been no specific study examining the impact of *T. angustifolia* and its removal on the re-establishment of wild rice.

The objectives of the study were to i) evaluate the effectiveness of mechanical removal of cattails as a method for restoring wild rice habitat; ii) to examine the impact of invasive cattails on long-term nutrient dynamics in a wild rice stand

1.2 Materials and Methods

1.2.1 Study Area

The study area, of Rat River Bay (48°37'19.80"N, 92°39'14.31"W) is 35km from the community. Rat River Bay runs north to south covers an of area of approximately 4.62km^2 and flows into Rainy Lake. It's a shallow waterbody with a maximum depth of approximately 4m with high clay mineral soil. Water level is controlled by the International Joint Commission (IJC) for power generation. It's entire littoral region is open water marsh, where *T. angustifolia* invasion begun in the early 1990's and has since create an extensive near monodominant stand used in our study and hence referred to as CT, interspersed with minimal remaining natural stands of wild rice referred to as WR.

This field experiment took place in a freshwater marsh belonging to the Seine River First Nations (SRFN). A formerly nearly monospecific stand of *Z. palustris* is now being invaded by *T. angustifolia*.

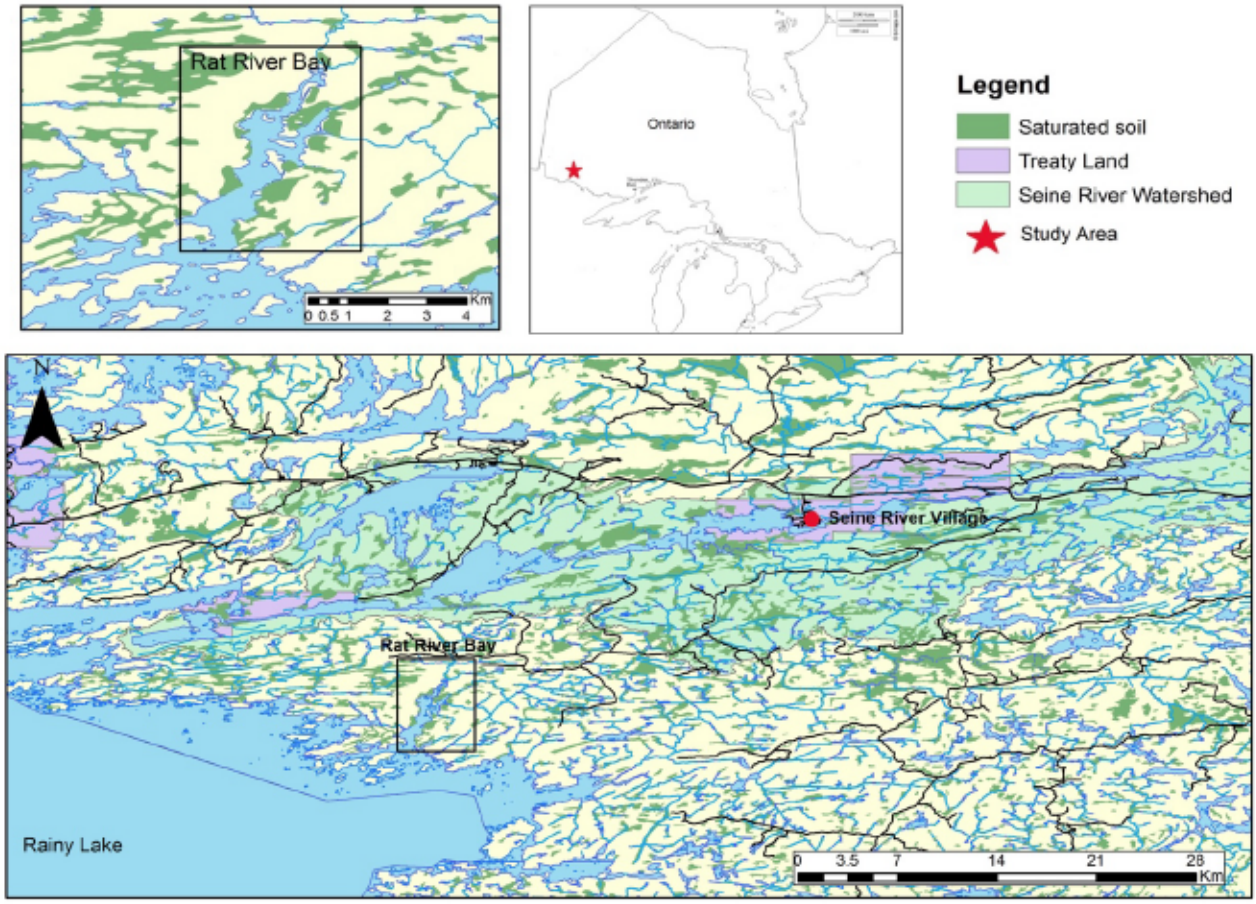

Figure 1. Location of Rat River Bay (48°37'19.80"N, 92°39'14.31"W) and proximity to Seine River Village in Northwestern Ontario.

1.2.2 Field Procedures

1.2.2.1 Mechanical Removal of Cattails

In September 2014, a cattail dominated site 25 x 50m^2 in Rat River Bay was selected for cutting. A sickle bar attached to an airboat was lowered into the water preforming a single cut of the stocks just above the sediment:water interface. Some biomass washed to shore within the cut site but most was removed from the system as it was washed downstream. The cut area is hence referred to as treatment site (TWR).

Figure 2. Cutting of Cattails in Rat River Bay by Seine River Community Members in September of 2014.

1.2.2.2 Temperature Logging

In May of 2017 nine Onset HOBO pendent (Model UA-002-64) temperature loggers were deployed in Rat River Bay, and recorded data hourly. They were placed on the water:sediment interface in three locations; a wild rice dominated site, a cattail dominated site and an open water

site. To reduce the confounding effects of water depth I targeted areas that had similar water depths. They were collected September 9, 2017 and data was offloaded using HOBO software (HOBOware 3.7.10).

1.2.2.3 Porewater Sample Collection

Dialysis pore water samplers (peepers) were deployed to collect sediment pore water samples as described by Jorgenson (2013) and shown in Appendix B.1. The framework is constructed of acrylonitrile butadiene styrene (ABS) pipes with three individual sample tubes in every 10 cm interval. Fisherbrand® 50 mL sample tubes were modified by drilling a 19 mm diameter hole in the cap and replaced with a 0.45 µm pore size Millipore Durapore® membrane filter. At time of deployment on September 9, 2015 sample tubes were filled with degassed distilled deionized water (DDW), capped with zero head space, and placed within the ABS pipe structure. The peepers were pushed vertically into the sediment with the top 10 cm interval visible the above the sediment water interface. In total, 6 peepers were deployed two in each study area (cattail dominated, wild rice natural stand, and treated area). After deployment, all peepers remained undisturbed for 35 days allowing adequate equilibrium (Teasdale et al. 1995). On October 14, 2015 the peepers were pulled vertically out of the sediment. Compromised or damaged samples were discarded and all three tubes from each depth were composited into one sample, collecting 150 mL of water at 10 cm interval. The samples were placed in ice filled cooler and transported to the Lakehead University Environmental Laboratory (LUEL).

1.2.2.4 Plant Density, Sediment and Plant Tissue Collection

The samples were collected in August of 2015 and 2017 along triplicate transects that ran perpendicular to the shoreline. In each transect, three 0.25m^2 sampling quadrats were selected based on length of transect (the depth of vegetation present). In each quadrat, water depth, cattail and wild rice height from sediment water interface to tallest leaf and stem density were measured, plants were then severed at the sediment:water interface for biomass measurement and chemical tissue analysis. In the same quadrat, sediment was collected by coring the top 20 cm. Both sediment and plant tissue were placed in cooler and immediately transported back to Lakehead University Environmental lab (LUEL) for analysis.

1.2.3 Laboratory Procedures

All sample analyses were conducted at LUEL, a Canadian Association of Laboratory Accreditation (CALA) ISO 17025 accredited laboratory. Quality was assured as analyses followed standard operation procedures which included the use of blanks, quality control samples, and duplicates.

1.2.3.1 Porewater Analysis

Water samples were mixed, allowed to settle for 5 to 10 minutes and then filtered with 0.45 um. Surface and pore water samples were analyzed for P (total P and phosphate), N (nitrite, nitrate, ammonia, total N) dissolved organic carbon (DOC), and total Al, As, Ba, Ca, Fe, K, Mg, Mn, Na, S, Sr and Zn.

Total N and P were analyzed by colourimetry using a SKALAR AutoAnalyzer®. Anions (Cl NO_2, NO_3, SO_4) were measured using Dionex 1100 ion chromatograph and ammonia was measured with a Dionex 120 ion chromatograph. Dissolved Organic Carbon was quantified by acidifying the sample and filtering it through a carbon dioxide permeable membrane prior to analysis on a SKALAR AutoAnalyzer®. Following the addition of HNO_3, water samples were digested and concentrated by microwave and analyzed by ICP spectrometry for Al, As, Ba, Ca, Fe, K, Mg, Mn, Na, S, Sr and Zn.

1.2.3.2. Sediment Analysis

Both total and extractable elemental analysis were conducted. All sediment samples were homogenized into uniform samples prior to analysis.

Sediment moisture was determined as the difference between the wet and dry weight and expressed in % of wet weight. The sediment samples were oven-dried at 105°C until a constant weight. Sediment bulk density of wet sediment was expressed as dry weight by volume (g/cm^3). Organic matter was determined by ashing sediment at 575°C until a constant weight was achieved. Organic matter content was calculated by weight difference between oven dried and ashed weights. Sediment pH and conductivity were determined using a 1:1 sediment to water ratio and measured on a Fisher accument XL 200 instrument.

Total concentrations of nutrient elements, were determined on dried sediment, ground to pass through a 2 mm mesh and homogenized into uniform samples. Samples were digested by microwave after the addition of HCl and HNO_3. Total P, Al, As, Ba, Ca, Fe, K, Mg, Mn, Na, S,

and Zn analyses were conducted by ICP spectrometry. Total carbon, total nitrogen and total organic carbon was were analysed with the Elementar Vario Cube analyzer (CHNS analyzer).

Available Ca, Mg, K and Na were determined following the extractions on wet sediment with ammonium acetate solution (pH = 7) while Fe, Mn, Cu and Zn were extracted in a 0.1N HCl solution. Both cations and metals were determined by ICP. The available N as ammonia (NH4-N) and nitrate was extracted with 1.0M KCl solution and using colorimetry and cadmium reduction on the SKALAR AutoAnalyzer®. The available phosphorus in the sediment was determined using the BRAY P1 method (Bray & Kurtz, 1945) whereby NH4F dissolves Al and Fe phosphates and forms complexes with these metals in acid solution. P was then measured by ICP.

1.2.3.3 Vegetation Analysis

Plants samples from each quadrat were weighed, and number of tillers/culms counted. All plant tissue samples were air dried and ground to pass through a 2 mm mesh then analyzed for Al, Ba, Ca, Fe, K, Mg, Mn, Na, S, Si, Sr, Ti and Zn using ICP spectrometry following a digestion and concentration by microwave and addition of HNO_3. Total carbon and nitrogen were determined using an Elementar Vario Cube analyzer (CHNS analyzer).

1.2.4 Data Analysis

Since our three sites (CT, WR, TWR) constituted the experiment unit of interest, I averaged the data collected from triplicate quadrats from each of the three transects (n=9). Skewness and kurtosis statistics were calculated for all variables to detect departures from the normal frequency

distribution assumed for most statistical tests. Square-root transformations were performed on dry weight per plant, stem density, and biomass (g/m2), plant tissue was not transformed. A Two-way ANOVA analyses was performed with sampling date (2015, 2017) and site (WT, TWR, CT) as factors for plant tissue. Variables were transformed to improve their normality, when assumptions were not meet analysis proceeded with caution (Green 1979). I utilized a repeated-measures analysis of variance (ANOVA) to compare water temperature between sites. A one-way ANOV was also performed for soil physiochemical properties. This was followed by a stepwise discriminant analysis to select variables that best discriminated among the sites. Since variables correlated to those selected by this procedure would not be included in further selections the correlation matrix of variables was examined to interpret the meaning of the derived discriminant functions. Wilks λ was used as the separating statistic among the environmental region centroids with the probability for inclusion of variables set at $P< 0.05$. Using the bivariate distribution which included 86.5 % in 2016 and 66.7 % of within group observation. All post hoc pairwise comparisons were based on Least Significant Paired comparison test (LSD). All statistics were run using SPSS version 25 with statistical significance of $p < 0.05$.

1.3 Results

1.3.1 Mechanical Removal

In the summer of 2014, the experimental site at Rat River Bay, was completely cattail dominated (Figure 3A). The one-time submerged cutting of invasive cattails in the fall of 2014 resulted in zero regrowth of cattails in 2015 (Figure 3B) and the successful volunteer re-establishment of wild rice (Figure 3C) from the pre-existing wild rice seed bank.

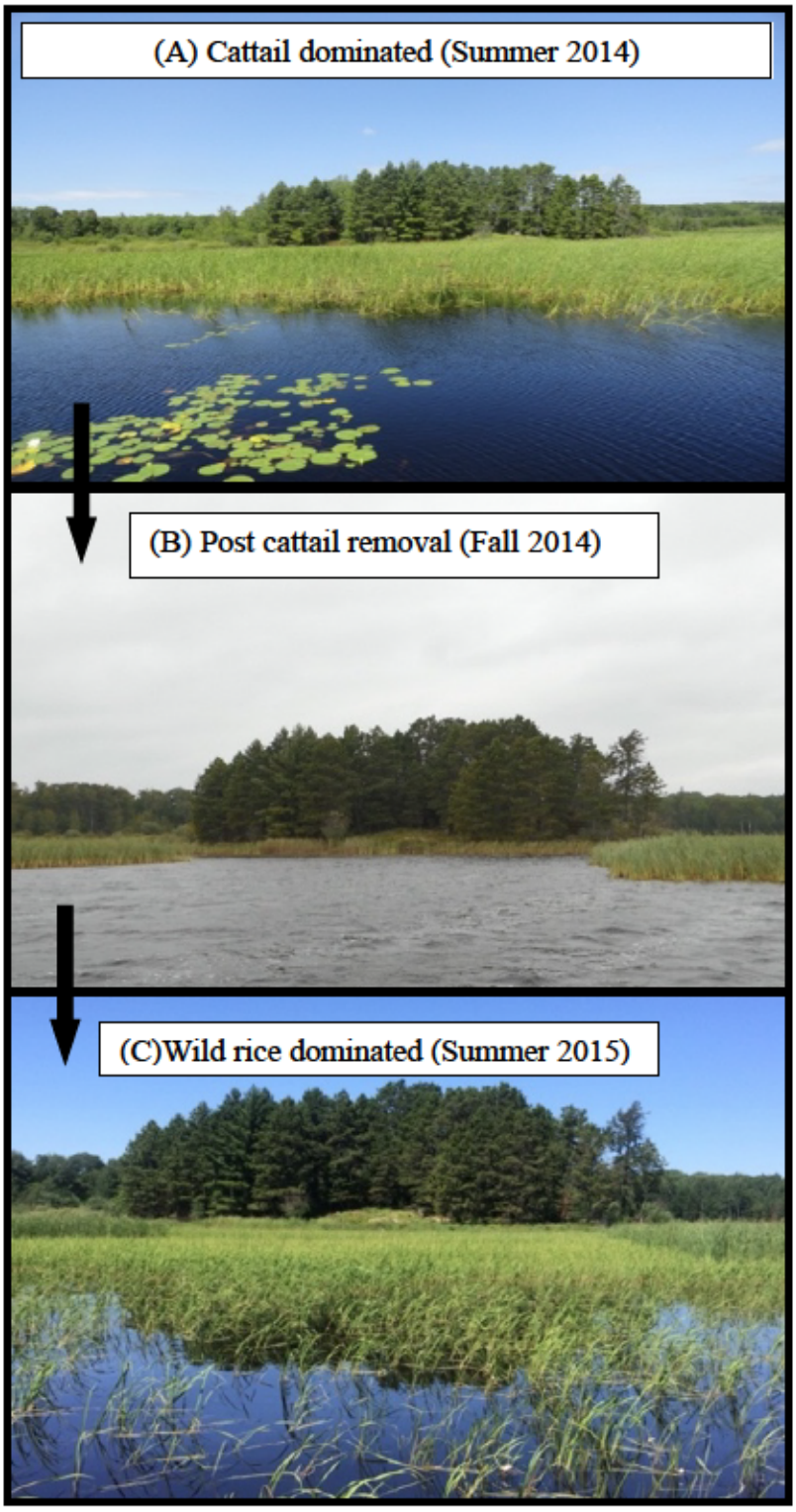

Figure 3. The treatment area in Rat River Bay. A: pre-treatment T. angustifolia dominated 2014, B: post cattail removal 2014, C: wild rice re-establishment 2015

1.3.2 Biomass and Density

There were no significant changes in cattail and wild rice stem density, weight per plant and biomass within sites between 2015 and 2017 (Figure 4). The cattail site, when compared to wild rice in natural stands, had significantly lower stem density ($P_{CT\ vs\ WR} = 0.004$) compared to natural wild rice stands (Figure 4A). However, plants were 4x larger ($P_{CT\ vs\ WR} < 0.001$) and therefore invasion increased biomass by over 2x that of the natural wild rice site ($P_{WR\ vs\ CT} < 0.001$)(Figure 4C).

In 2015, wild rice in the treatment site had twice the density of that in the natural stand ($P_{WR\ vs\ TWR} < 0.001$) while the difference in density reduced to 14% in 2017 (Figure 4A). Also, treated wild rice had significantly less weight in 2015 ($P_{WR\ VS\ TWR} =< 0.001$), but there was no longer a significant size difference in 2017 (Figure 4B). Consequently, neither year resulted in a significant difference in biomass ($P_{WR\ VS\ TWR} = 0.102$) between natural and treated sites (Figure 4C).

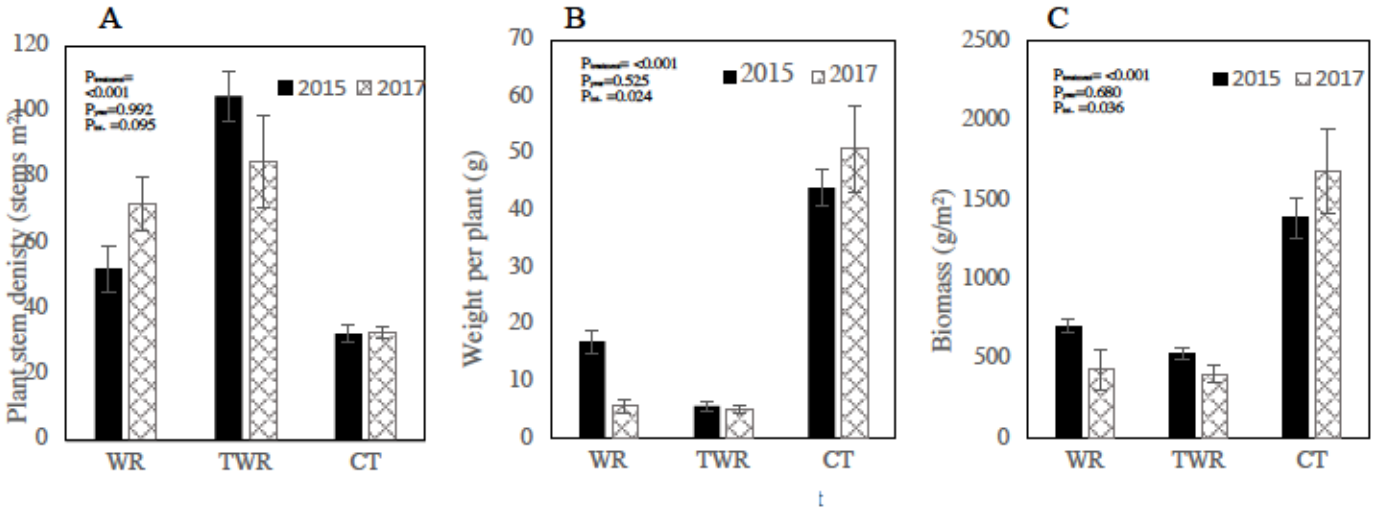

Figure 4. Mean value of A) Plant density B) weight per plant C) Biomass from treatment sites. Wild rice natural stands (WR) Wild rice from treatment site (TWR) and cattails (CT). Two-way ANOVA was used to determine significant differences with sites and year.

1.3.3. Temperature Data

Water temperature was higher in the wild rice site throughout the entire growing season, while the cattail dominated site was the coldest (Figure 5).

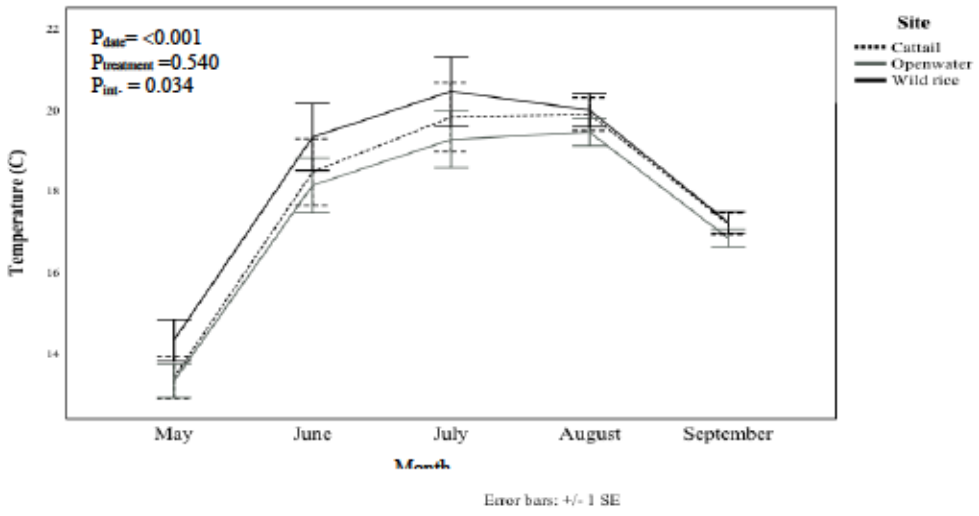

Figure 5. Average daily water temperature from May to September of 2017. Water temperatures from HOBO dataloggers placed on the water sediment interface in a Wild rice dominated site, Cattail dominated site and Open water site. Repeated measures analysis was used to determine the significance between treatments and dates

1.3.4 Plant Tissue

Nutrient analysis of plant tissue from a wild rice site (WR), treated site (TWR) and cattail site (CT) is summarized in Table 1. The comparison of native wild rice versus cattail showed that cattails had a significantly higher concentration of carbon ($P_{CT\ vs\ WR} = < 0.001$), manganese ($P_{CT\ vs\ WR} = < 0.001$), barium ($P_{CT\ vs\ WR} = 0.019$), C:N ($P_{CT\ vs\ WR} = 0.002$), and C:P ratio ($P_{CT\ vs\ WR} = < 0.001$). Tissue of wild rice in natural stands have a significantly higher concentration of aluminum ($P_{CT\ vs\ WR} = < 0.001$), calcium ($P_{CT\ vs\ WR} = < 0.005$), iron ($P_{CT\ vs\ WR} = < 0.001$), potassium ($P_{CT\ vs\ WR} = < 0.001$), phosphorus ($P_{CT\ vs\ WR} = < 0.001$), sulfur ($P_{CT\ vs\ WR} = < 0.001$), and nitrogen ($P_{CT\ vs\ WR} = < 0.001$) than wild rice. Copper, Magnesium, nickel, Zinc, are not significantly different. When comparing the tissue concentration of wild rice in natural stand to wild rice in treatment site, natural stands had higher potassium ($P_{WR\ vs\ TWR} = 0.010$), carbon ($P_{WR\ vs\ TWR} = 0.038$) and nitrogen ($P_{WR\ vs\ TWR} = 0.03$) but lower in sodium ($P_{WR\ vs\ TWR} = 0.006$) and zinc ($P_{WR\ vs\ TWR} = 0.018$) (Table 1).

Table 1. Average and standard deviation of plant tissue results from Cattail (CT) plant tissue and Wild rice tissue from natural stand (WR) and treated sites (TWR). One-way ANOVA was used to determine significant difference between plant tissue from study sites (n= 20 WR, n= 16 TWR, n=12 in CT for 2015; n=6 in WR, TWR, CT in 2017). Bolded figures indicate those variables that varied significantly ($P < 0.05$), with different letters used to indicate significant differences between sites

Parameter	WR		TWR		CT	
	Average	**Sd**	**Average**	**Sd**	**Average**	**Sd**
Aluminum (µg/g)	**153.3a**	**122.7**	**122.7a**	**76.9**	**32.9b**	**16.1**
Barium (µg/g)	**11.6a**	**3.3**	**11.9a**	**2.6**	**14.0b**	**3.5**
Calcium (%)	**0.8a**	**0.3**	**0.9a**	**0.4**	**0.5b**	**0.1**
Copper (µg/g)	3.2	0.8	3.1	1.3	3.4	0.5
Iron (µg/g)	**444.1a**	**264.3**	**342.4a**	**121.6**	**108.4b**	**81.4**
Potassium (%)	**1.3a**	**0.3**	**1.1b**	**0.3**	**0.8c**	**0.3**
Magnesium (%)	0.26	0.1	0.22	0.1	0.3	0.1
Manganese (µg/g)	**122.3a**	**82.5**	**125.9a**	**40.7**	**438.1b**	**243.3**
Sodium (µg/g)	**3174.1ac**	**1526**	**4634.4b**	**2341**	**2258.6c**	**1067**
Nickel (µg/g)	1.1	1.4	1.5	2.1	1.8	2.2
Phosphorus (%)	**0.22a**	**0.1**	**0.2a**	**0.1**	**0.2b**	**0**
Sulphur (%)	**0.19a**	**0.1**	**0.17a**	**0.1**	**0.1b**	**0**
Zinc (µg/g)	**12.5ac**	**1.9**	**14.2b**	**3.1**	**12.3c**	**1.9**
Carbon (%)	**41.1a**	**0.8**	**40.6b**	**1.1**	**44.6c**	**5.1**
Nitrogen (%)	**2.1a**	**0.7**	**1.6b**	**0.5**	**1.4b**	**0.4**
C:N	**22.4a**	**11.9**	**26.6ab**	**10.2**	**32.9b**	**6.8**
C:P	**209.8a**	**82.9**	**216.5a**	**59.3**	**289.1b**	**42.5**

Due to the significant difference in biomass between study sites (Figure 4C) to accurately assess the impact of invasion and removal of cattails the plant tissue concentrations were converted to mg per m^2 (Figure 6) using the biomass values determined in field quadrats.

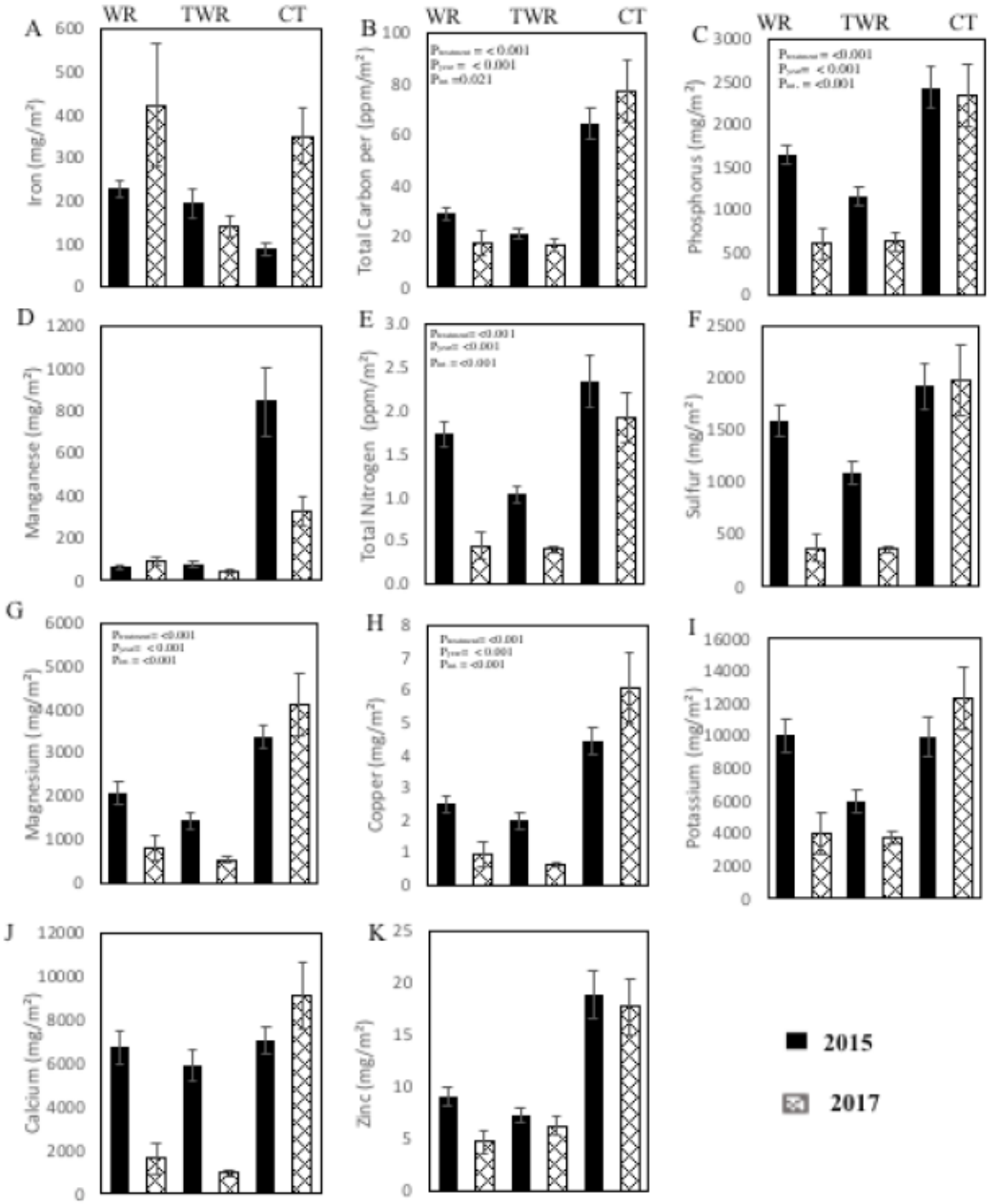

Figure 6. Two way-ANOVA results with average plant tissue between sites wild rice (WR), TWR- Treated site (TWR), cattail (CT), and years (2015, 2017).

Invasive cattail sites had significantly higher uptake of phosphorus ($P_{CT\ vs\ WR} = < 0.001$), potassium ($P_{CT\ vs\ WR} = 0.029$), carbon ($P_{CT\ vs\ WR} = < 0.001$), calcium ($P_{CT\ vs\ WR} = 0.001$), zinc ($P_{CT\ vs\ WR} = < 0.001$), magnesium ($P_{CT\ vs\ WR} = < 0.001$), manganese ($P_{CT\ vs\ WR} = < 0.001$), and sulfur ($P_{CT\ vs\ WR} = < 0.001$) (Figure 6).

Uptake by wild rice in the treatment site was significantly lower in total nitrogen ($P_{WR\ vs\ TWR} < 0.001$), phosphorus ($P_{WR\ vs\ TWR} = 0.011$) and potassium ($P_{WR\ vs\ TWR} = 0.001$) when compared to natural stand (Figure 6).

Noteworthy is the depletion of micro and macronutrients within the treatment site three years post-removal. This includes, phosphorus ($P_{2015\ vs\ 2017} = < 0.001$), carbon ($P_{2015\ vs\ 2017} < 0.001$), nitrogen ($P_{2015\ vs\ 2017} = < 0.001$) , potassium ($P_{2015\ vs\ 2017} = < 0.001$), calcium ($P_{2015\ VS\ 2017} = 0.000$), zinc ($P_{2015\ VS\ 2017} = 0.004$), sulfur ($P_{2015\ vs\ 2017} = < 0.001$) , magnesium ($P_{2015\ VS\ 2017} = < 0.001$), copper ($P_{2015\ VS\ 2017} = < 0.001$), and iron ($P_{2015\ VS\ 2017} = 0.001$) (Figure 6).

Wild Rice Chlorosis

The colouration of wild rice plants within natural wild rice stands and wild rice within treated areas was noticeably different. Wild rice from the natural wild rice site had the outward appearance of healthier plants with less disease and a deeper green colour (Figure 7A). The wild rice plants from the treatment site with symptoms of nitrogen deficiencies (Day and Lee, 1990) showing a yellow chlorotic appearance and abundant brown spots (Figure 7B).

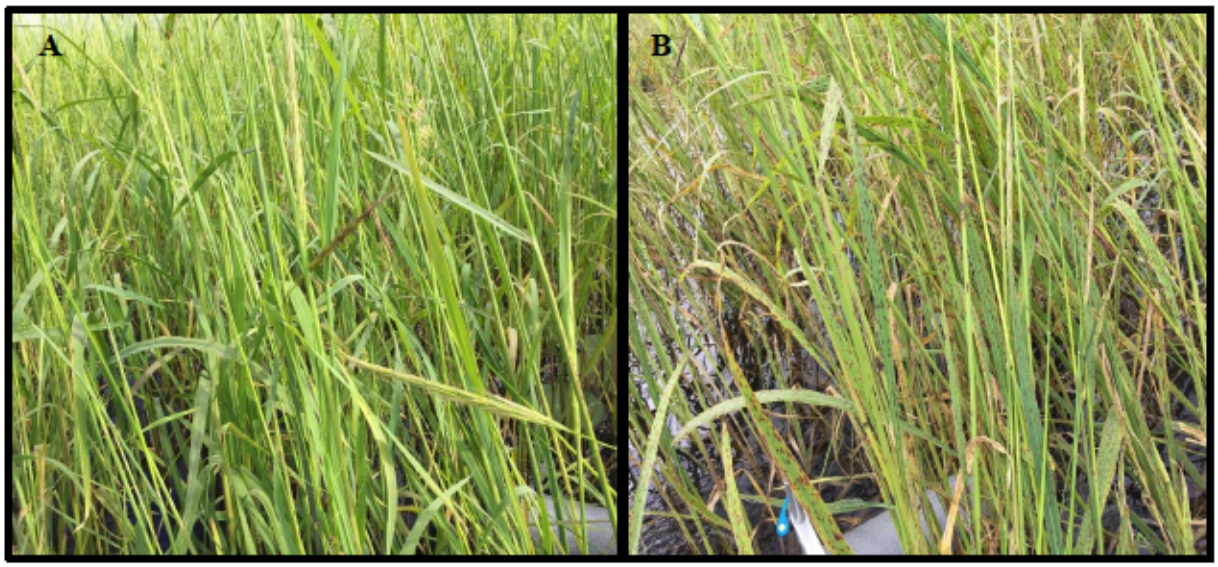

Figure 7. In the WR site note the general healthy look of the plants with lower incidence of brown spots on leaves and stems of plants. This was a general observation made throughout the natural stand B: Wild rice plants within TWR. Note high incidence of brown spots on leaves and stems of WR plants. Similar results occurred throughout the treated area.

1.3.5 Sediment and Porewater

Analysis of porewater within the root zone (0 to 20 cm), revealed cattail dominance and mechanical removal treatment significantly impacted nutrient concentrations of porewater. The results show the average concentrations and standard deviations for the natural wild rice site, the treated wild rice site and cattail dominated site (Table 2).

There was no significant difference in porewater concentrations between sites for dissolved organic carbon, nitrate, iron, potassium, manganese, sodium, zinc, phosphates, and sulphates total nitrogen and total phosphorus. Cattail dominance resulted in a significantly lower ammonium ($P_{WR\ vs\ CT} = 0.021$), chloride ($P_{WR\ vs\ CT} = 0.002$), calcium ($P_{WR\ vs\ CT} = 0.012$), and magnesium ($P_{WR\ vs\ CT} = 0.08$). Removal treatment significantly reduced chloride ($P_{WR\ vs\ TWR} = 0.010$), and sulphur ($P_{WR\ vs\ TWR} = 0.001$) concentration in the root zone of the sediment.

Table 2. Average porewater analytical data collected in 2015 from Cattail dominated site (CT) a wild rice from a natural stand (WR) and treated site (TWR). One-way ANOVA was used to determine significant difference between study sites (n= 8). Bolded figures indicate those variables that varied significantly ($P < 0.05$), with different letters indicating significant differences between sites.

Parameters	WR		TWR		CT	
mg/L	Average	Sd	Average	Sd	Average	Sd
Dissolved Organic Carbon	19.3	2.8	13.9	4.6	16.82	3.9
Chloride	**0.6a**	**0.2**	**0.2b**	**0.1**	**0.1b**	**0.0**
Ammonium	**0.4a**	**0.2**	**0.2ab**	**0.3**	**0.1b**	**0.1**
Nitrate	0.01	0.0	0.010	0.1	0.005	0.0
Calcium	**13.4a**	**3.6**	**9.8ab**	**1.7**	**7.9b**	**1.3**
Iron	4.5	3.9	0.8	0.9	2.9	1.5
Potassium	0.3	0.3	0.3	0.2	0.4	0.2
Magnesium	**5.9a**	**1.4**	**4.8ab**	**0.9**	**3.4b**	**0.5**
Manganese	0.4	0.3	0.2	0.1	0.2	0.1
Sulphur	**0.3a**	**0.0**	**0.2b**	**0.1**	**0.3a**	**0.0**
Zinc	0.005	0.0	0.004	0.0	0.007	0.0
Phosphates	0.12	0.1	0.05	0.1	0.13	0.1
Total Phosphorous	0.11	0.1	0.04	0.1	0.12	0.1
Total Nitrogen	1.22	0.2	1.01	0.2	0.99	0.1

The results of sediment chemical analysis for total values are summarized in Table 3 and extractables values in Table 4. The impact of invasion was assessed by comparing the sediment from a natural wild rice stand to a cattail dominated site, which significantly decreased phosphorus ($P_{CT\ VS\ WR} = 0.016$) in 2015, and manganese ($P_{CT\ VS\ WR} = 0.012$) in 2017. Treatment did not significantly change any variable in 2015 and 2017.

Table 3. Total Sediment variables mean and standard deviation samples in 2015 and 2017 at a cattail dominated site (CT) a wild rice from a natural stand (WR) and treated site (TWR). One-way ANOVA was used to determine significant differences between study sites (n= 20 WR, n= 15 in TWR, n=14 in CT for 2015; n=6 WR, TWR, CT in 2017). Bolded figures indicate those variables that differed significantly ($P < 0.05$), with different letters indicating significant differences between sites.

	2015						2017					
	WR		TWR		CT		WR		TWR		CT	
	Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd
Total Al g/m²	956.8	534.3	818.0	549.9	1173.9	730.3	1909.1	969.3	1624.2	274.5	1074.6	547.6
Total Ba g/m²	7.7	4.5	7.1	5.6	11.9	10.2	16.3	9.9	12.0	3.0	7.6	4.2
Total Ca g/m²	376.3	120.8	405.1	167.3	497.0	213.1	558.2	211.6	623.8	239.8	513.5	287.9
Total Co g/m²	0.4	0.3	0.3	0.2	0.5	0.3	0.9	0.5	0.5	0.1	0.5	0.3
Total Cr g/m²	2.0	1.3	1.8	1.5	3.1	3.0	4.3	2.4	3.1	0.6	2.6	1.6
Total Cu g/m²	3.1	1.5	2.5	1.8	4.0	2.5	5.6	2.8	6.1	1.1	3.5	2.1
Total Fe g/m²	625.3	543.7	493.3	366.2	792.8	601.8	1386.9	766.4	871.6	240.6	836.9	499.3
Total K g/m²	45.4	33.7	33.6	24.5	47.5	23.7	76.2	42.6	45.3	13.1	35.6	16.5
Total Mg g/m²	249.9	214.5	252.4	192.3	363.2	219.3	597.2	329.3	385.3	86.5	345.9	190.0
Total Mn g/m²	8.2	8.0	6.1	3.7	8.5	6.7	**15.9a**	**9.4**	**8.3ab**	**2.2**	**8.3b**	**3.1**
Total Na g/m²	156.1	71.7	119.5	60.9	165.2	84.5	13.1	5.5	9.5	2.8	6.6	3.0
Total Ni g/m²	2.1	1.1	1.8	1.2	3.1	2.4	4.0	1.8	4.1	1.0	2.6	1.2
Total P g/m²	**61.7a**	**15.50**	**53.0ab**	**14.10**	**46.2b**	**10.00**	74.5	29.2	96.0	36.5	74.5	40.8
Total Pb g/m²	0.5	0.3	0.4	0.2	0.6	0.4	1.2	0.7	0.8	0.3	0.7	0.5
Total S g/m²	103.1	17.7	90.4	13.9	88.7	19.5	109.0	39.2	146.0	68.5	160.3	59.3
Total Si g/m²	16.3	13.7	9.7	5.3	10.9	6.0	350.1	192.3	261.9	33.3	189.2	96.8
Total Sr g/m²	1.3	0.4	1.2	0.4	1.8	1.4	2.2	1.0	2.0	0.5	1.7	0.8
Total Zn g/m²	2.1	2.6	1.8	1.8	2.6	1.7	5.3	3.3	2.6	0.9	2.8	1.7
TOC g/m²	8271.5	1833.5	7020.6	1092.8	6770.8	1159.2	-	-	-	-	-	-
Total C g/m²	8583.9	1885.4	7414.0	1082.0	7090.9	1197.1	9465.6	2787.8	13324.4	4965.1	13975.0	7715.4
Total N g/m²	735.0	136.3	631.2	103.0	598.1	121.4	849.3	261.0	1175.8	373.6	1120.1	634.0

Summarized in Table 4 are the results of the sediment nutrient analysis for extractable variables. In 2015, cattail dominance resulted in significantly increased extractable calcium ($P_{CT\ vs\ WR}$ =0.013) , extractable potassium ($P_{CT\ vs\ WR} = 0.014$), extractable copper ($P_{CT\ vs\ WR} = 0.04$), and extractable zinc ($P_{CT\ vs\ WR}$ =0.020), but depleted soil moisture ($P_{CT\ vs\ WR} = 0.005$), pH ($P_{CT\ vs\ WR}$ =< 0.001), available N ($P_{CT\ vs\ WR}$ = < 0.001), and extractable ammonium ($P_{CT\ vs\ WR}$ = < 0.001). In 2017, cattail site was higher in moisture ($P_{WR\ vs\ CT}$=0.018), LOI ($P_{CT\ vs\ WR}$=0.012) and redox ($P_{CT\ vs\ WR}$= 0.014) but significantly depleted extractable manganese ($P_{WR\ vs\ CT}$= 0.015). In 2015, the removal of cattails significantly depleted extractable ammonium ($P_{TWR\ vs}$

$_{WR}$ = < 0.001), available N ($P_{TWR\ VS\ WR}$ = < 0.001), and increased pH ($P_{TWR\ VS\ WR}$ = < 0.001. In 2017, the only difference due to treatment is the depletion in extractable manganese ($P_{WR\ VS\ CT}$ =0.027) (Table 4).

Table 4. Extractable sediment variables mean and standard deviation samples in 2015 and 2017 at a cattail dominated site (CT) a wild rice from a natural stand (WR) and treated site (TWR). One-way ANOVA was used to determine significant difference between study sites (n= 20 WR, n= 15 in TWR, n=14 in CT for 2015; n=6 WR, TWR, CT in 2017). Bolded figures indicate those variables that varied significantly (P < 0.05), with different letters used to indicate significant differences between sites

	2015						2017					
	WR		TWR		CT		WR		TWR		CT	
	Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd	Mean	Sd
Ext. Ca g/m2	**163.8a**	**48.8**	**202.2ab**	**81.1**	**250.3b**	**108.2**	307.1	137.0	300.4	80.8	234.9	125.8
Ext. K g/m2	**1.3a**	**1.3**	**1.2ab**	**0.6**	**2.3b**	**2.0**	16.8	21.3	5.1	4.0	3.3	1.1
Ext. Mg g/m2	48.0	19.4	57.9	33.1	67.6	26.6	117.1	62.7	91.0	17.5	62.5	35.8
Ext. Na g/m2	1.0	0.4	0.8	0.5	4.1	9.1	3.0	1.7	1.9	0.6	1.5	0.6
Ext. Cu g/m2	**0.3a**	**0.2**	**0.3a**	**0.1**	**0.5b**	**0.3**	0.7	0.7	0.3	0.1	0.2	0.2
Ext. Fe g/m2	13.0	7.0	11.0	7.8	21.7	22.9	39.5	29.4	17.2	10.3	19.9	14.4
Ext. Mn g/m2	2.1	1.4	1.5	0.8	2.2	2.2	**6.4a**	**3.9**	**2.8b**	**0.7**	**2.6b**	**0.8**
Ext. Zn g/m2	**0.2a**	**0.2**	**0.2a**	**0.1**	**0.3b**	**0.1**	0.5	0.4	0.20	0.1	0.4	0.2
Ext. NH3 g/m2	**0.5a**	**0.3**	**0.2b**	**0.1**	**0.2b**	**0.2**	-	-	-	-	-	-
Ext. NO3 g/m2	0.30	0.3	0.20	0.2	0.17	0.3	-	-	-	-	-	-
Avail. N g/m2	**0.8a**	**0.4**	**0.4b**	**0.2**	**0.3b**	**0.3**	5.4	1.3	5.4	1.6	5.3	2.3
Ext. P g/m2	1.8	1.4	2.7	1.6	1.7	1.3	2.7	1.6	2.2	0.7	3.1	1.6
Bulk Density	0.2	0.1	0.2	0.1	0.3	0.1	0.5	0.2	0.4	0.0	0.3	0.1
Conductivity	69.8	22.9	63.9	26.0	69.9	27.4	62.9	8.8	64.8	22.4	52.4	31.7
% Moisture	**50.1a**	**4.7**	**51.7a**	**3.6**	**45.2b**	**5.2**	**37.6a**	**7.4**	**41.8ab**	**2.4**	**44.8b**	**2.2**
Lost On Ignition	-	-	-	-	-	-	**31.7a**	**19.9**	**48.1ab**	**18.8**	**64.6b**	**26.6**
Redox Potential	-	-	-	-	-	-	**67.8a**	**17.8**	**69.7a**	**12.6**	**95.1b**	**11.5**
pH	**5.6a**	**0.1**	**5.9b**	**0.2**	**5.9b**	**0.2**	5.6	0.2	5.7	0.1	5.6	0.2

1.3.6 Discriminant Analysis

Table 5 indicates the relative significance of the variables comprising the two discriminant functions in 2015. The first function, which explained 83.4% of the separation of groups, was composed mostly of the pH and % moisture, relative to extractable ammonia. The second

function which explained 16.6% of the group separation was also composed mostly of pH and % moisture and a weak positive correlation to extractable ammonia.

Examination of the correlation matrix showed that moisture was highly correlated to total carbon (r=0.741), total nitrogen (r=0.759) and negatively correlated to bulk density (r=-0.729) and TOC (r=-0.730) and extractable ammonia was positively to extractable iron (r=0.559). The discriminating variable of pH is positively correlated to total magnesium (r=0.701), total manganese (r=0.682), total nickel (r=0.682), total iron (r=0.666), total potassium (r=0.653) and negative correlation to total carbon (r=-0.725) and total nitrogen (r=-0.723).

Table 5. Characteristic of discriminant functions (DF1, DF2) used to separate the wild rice, treated and cattail sites in 2015

Canonical Discriminant Function Coefficients		
Variable	DF1	DF2
Moist	0.548	1.203
Extractable Ammonia	-0.682	0.521
pH	1.056	0.522
Relative % explained	83.4	16.6
Function at group Centroids		
Wild rice	-1.842	0.123
Treated	2.024	0.705
Cattail	1.241	-1.643

* standardized coefficients

Figure 8 illustrates the separation of sites in 2015 with respect to their increased value of discriminant scores. Discriminant function 1 was primarily responsible for separating wild rice from cattail dominated site and treatment site. It could be interpreted as the nitrogen increases, organic content and lower pH found in the wild rice site. Discriminant function 2 separated cattail site from treated site. This function could be interpreted as increasing values of organic

content, N and pH found un the treated versus the cattail site. The wild rice site was the most distinct and separated from the other site with a 95% accuracy. The treated site was separated with an 86.7% accuracy but it's interesting to note it was equally misclassified as treated site and cattail site (6.7%). Cattail was the least distinct with a 58.3% accuracy, most often misclassified as treated site.

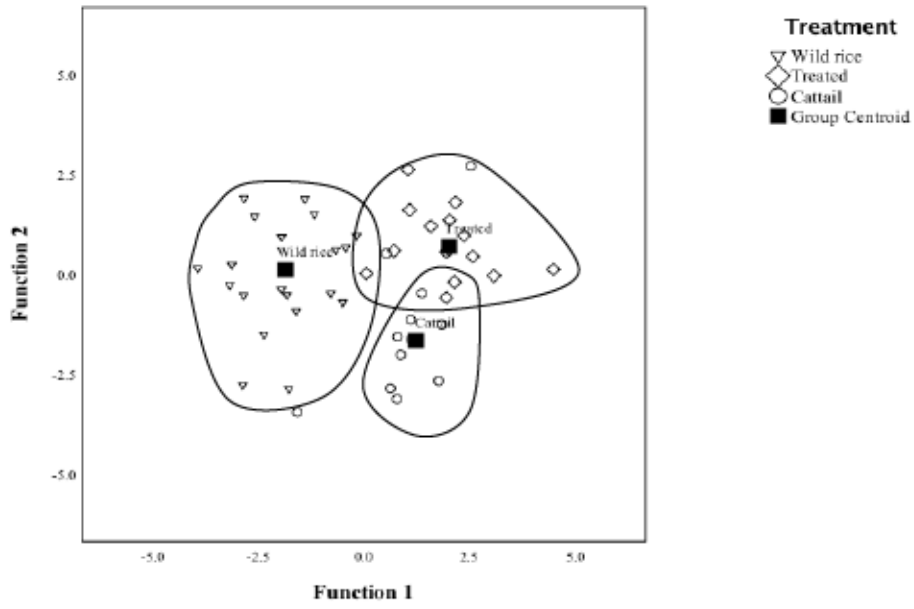

Figure 8. Canonical plot constructed through the stepwise discriminant analysis of 2015 sediment physiochemical analysis using Wilks λ as the separating statistic for the cattail dominated site, treatment site and the wild rice site.

Table 6 indicated the relative significance of the variables comprising the two discriminate functions in 2017. The first function, which explained 94.2% of the separation of the groups, was composed of the redox and extractable zinc. The second function which explained 5.8% of the group separation was also composed mostly extractable zinc with a negative correlation to redox. Examination of the correlation matrix illustrated that zinc was highly positively correlated to

extractable iron (r=0.703), extractable copper (r=0.767), extractable manganese (r=0.696), total magnesium (r=0.761), total iron (r=0.799), total zinc (r=0.880) and bulk density (r=0.738) and negatively correlated to redox (r=-0.697). The second discriminating variable, redox was positively correlated to total nitrogen (r=0.634) and total carbon (r=0.637), and negatively correlated to extractable copper (r= -0,702), extractable manganese (r= -0.707), extractable zinc (r=-0.697), total zinc (r= -0.759), total potassium (r= -0.766).

Table 6. Characteristic of discriminant functions (DF1, DF2) used to separate the wild rice, treated and cattail sites in 2017.

Canonical Discriminant Function Coefficients		
Variable	DF1	DF2
Extractable Zn	1.098	0.86
Redox	1.382	-0.188
Relative % explained	94.2	5.8
Functions at Group Centroids		
Wild rice	-0.563	0.415
Treated	-1.172	-0.328
Cattail	1.735	-0.087

* standardized coefficient

Figure 9 illustrates the separation of sites with respect to their discriminant scores. Discriminant function 1 was primarily responsible for separating wild rice from cattail and treated site. The second discriminant function separated wild rice from treated site. The cattail site was the most distinct and separated from the others sites with an 83.3% accuracy. The wild rice site was the least distinct and most often misclassified as a treated site. Wild rice was classified as a treated site at 33% of the time. Noteworthy, is the loss of distinction between sites from one-year post

removal to three years post-removal. The 2015, discriminant analysis accurately reclassified 88.9% while in the 2017 discriminant analysis reclassified with a 66.7% accuracy.

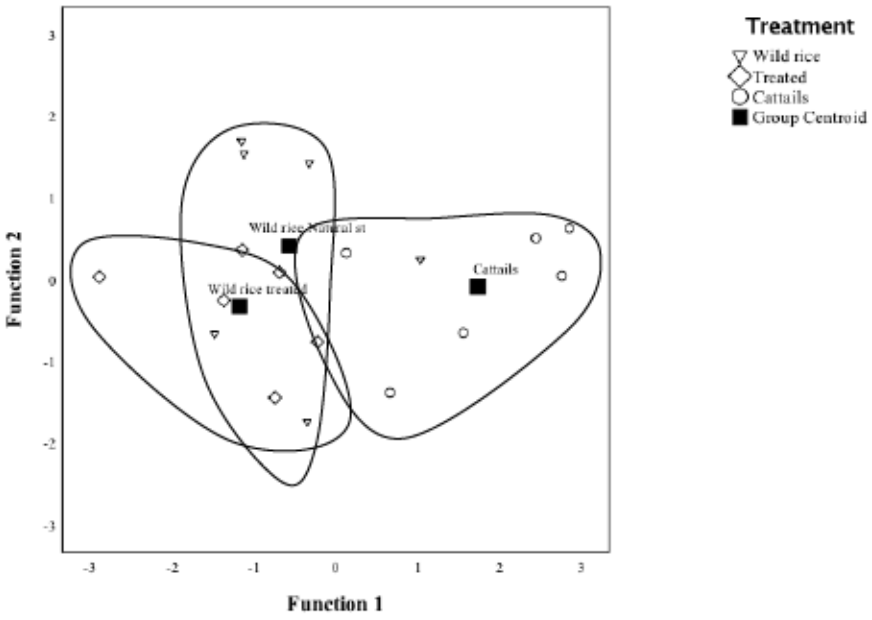

Figure 9. Canonical plot constructed through the stepwise discriminant analysis of sediment physiochemical analysis in 2017 using Wilks λ as the separating statistic for cattail dominated site, treatment site and the wild rice site.

1.4 Discussion

1.4.1 Cattail removal and wild rice re-establishment

Results of this study indicate that a single submerged removal of cattail stocks successfully eliminated the invasive cattail for at least 3 years, with zero cattail re-establishment within the treatment site (Figure 5). Results in Rat River Bay are comparable to those reported by Sale and Wetzel (1983), Jordan and Whigham (1988), Nelson and Dietz (1966), Zedler (2010), Singh at al. (1973), Hellsten et al. (1999), and Lishawa at al. (2017). Sale and Wetzel (1983) reported

rapid decomposition of nearly all underground rhizomes after cutting and is credited for success of methods. In contrast, Hall and Zedler (2010) and Keyport et al (2018) used similar methodology but reported that removal must be repeated every year to prevent cattail re-establishment.

Perhaps the most striking result of this study was the successful volunteer re-establishment of native wild rice. Very little is understood about the germination (Sims et al. 2012) and viability of wild rice (Atkins 1987). This study suggests that some factor of cattail dominance prevents the germination but retains viability of wild rice seed. Possibilities include the depletion of nitrogen (Sims et al. 2012), change in diurnal temperature fluctuation (Atkins,1987), change in oxygen tension contributes to secondary dormancy (Svare 1960, Simpson 1966), and possible allelopathic effects from cattail on wild rice germination (Jarchow and Cook 2009).

Important considerations in any restoration effort is the effectiveness and longevity of the chosen management strategy. Results of this study showed that wild rice biomass in the treatment site was sustained three years post-removal. Also encouraging is how the wild rice biomass did not differ from the natural stand (Figure 3). Wild rice plant weight and density for natural and treated wild rice is comparable to productive stands reported by Lee (1979). The scope of this study did not allow for continued monitoring, but results indicate the potential for long-term success. While establishing new stands of wild rice in new environments is possible, Keenan and Lee (1986) and Painchaud and Archibold (1990) reported varying degrees of success with seeding. Mechanical removal of invasive cattails from historic wild rice sites can significantly contribute to increased wild rice populations. Future work should consider the lack of knowledge

on seed bank ecology including what influences wild rice seed viability and secondary dormancy.

In Rat River Bay, the immense above ground biomass and stem density of invasive cattails (Figure 3) were comparable to data reported in other fresh water marshes in North America (Farnsworth and Meyerson 2003, Geddes et al. 2014, McNaughton 1966, Findlay et al. 2002). This ability to retain significant biomass over natives is of particular importance as it may contribute to its persistence and its superior competitive ability (Ehrenfeld 2003, Harper 1977). Data from sediment and plant tissue support this idea.

1.4.2 Impact of Microhabitat due to Invasion and Mechanical Removal Treatment

1.4.2.1 Temperature

The decrease in temperature is an import modification that occurs under cattail dominance (Figure 6). Temperature under cattail dominance was an average of 0.5 °C cooler a trend also reported by Angeloni et al. (2016), Larkin et al. (2011), Lawrence (2016) and Freyman (2008). The decrease in temperature can be attributed to the large size of the cattail, which increases shade. This temperature difference can stifle competition and slow decomposition causing a build-up of organic matter (Reddy and Delaune 2008). Furthermore, Clay and Oelke (1987) reported shading of wild rice by Giant Burreed (*Sparganium eurycarpum*) to be the major mechanism in which the invasive outcompetes wild rice. The decrease due to *Typha* invasion may impact postpone or supress the germination of native vegetation.

1.4.2.2 Plant Tissue

There were significant differences in plant tissue concentration (Table 7) and total uptake of nutrients (Figure 7) as a result of cattail invasion and removal treatment. The observed changes can be related to the difference between the native and invasive plant physiology (annual vs perennial), morphology, assimilation and decomposition, all of which play an important role in nutrient dynamic and availability (Whittaker 1965, Farnsworth and Meyerson, 2003). This perpetuated difference can modify an environment creating one that is distinctly different than what was seen under native vegetation, in this case wild rice (Ehrenfeld 2003, Lee and McNaugthon 2004, Callaway et al. 2004). Nutrient concentration is wild rice tissue are characteristic of growth in nutrient poor environments and are similar to Grava and Raisanen (1978) and Lee and McNaughton (2004).

Almost all macronutrients (N, P, K, Ca, S, C) had significantly lower in concentrations in cattail tissue (Table 1), but when you consider the significant difference between cattail and wild rice in total per unit area biomass all macronutrients experienced a significant increase in uptake in the cattail dominated site (Figure 7). Thus, the higher net primary productivity (Linde et al. 1976) contributes greatly to the retention of nutrients, a characteristic of many invasive species (Jordan et al. 1989, Ehrenfeld 2003). While, other nutrients (Zn, Mg, Mn) experienced higher levels of uptake in cattail dominated site, these could also be related to species specific accumulation magnified by difference in biomass (Figure 2). It's noteworthy that zinc, potassium, magnesium and manganese were noticeable higher in sediment of the cattail dominated site suggesting their greater uptake and retention repeated over multiple years or growth and decay lead to the creation of a distinctly different environment (Larkin et al. 2012).

The high nitrogen in natural wild rice plant tissue results in a low C:N ratio (Table 1) and high decay rate, which reduces net immobilization and leads to higher nitrogen levels in the soil (Reddy and Delaune 2008). In contrast, the high C:N ratio in cattails impedes breakdown of organic matter and can temporarily immobilize N in addition to reduced decay rates resulting in nutrient depletion and buildup of litter. When comparing annuals and perennials, consideration must be given to the translocation of nutrients from rhizomes that occurs in perennials which increases initial retention of these nutrients in their above ground plant tissue (Morris and Lajtha 1986).

Removal of cattails resulted in "less healthy" wild rice plants (Figure 7) that were significantly depleted in N, P, K with visible signs of nutrient stress (Day and Lee 1990). This suggesting that accumulation of available nutrients in invasive cattails resulted in a nutrient depletion for re-establishing native wild rice. Another notable trend seen in the wild rice tissue within the treatment site is the depletion of several micro and macro-nutrients (Figure 6) when comparing wild rice tissue from year one to year three post treatment. This may be explained by the increased nutrient availability provided by the decomposing rhizomes in the treatment site.

1.4.2.3 Sediment and Porewater

The comparison of soil and porewater characteristics illustrates that both cattail invasion of and removal have created distinctly different environments (Figure 8). Furthermore, the discriminant analysis suggests that the distinction between treatment site and the natural stand sites decreases over time (Figure 9). This indicates that dominate macrophyte is influencing the biogeochemistry

of sediment and porewater (Templer et al. 1998, Meyerson et al. 1999,) Thompson et al. 2009) as the re-establishment of wild rice within the treatment site resulted in little distinction with the pre-existing natural stands.

Nitrogen may be a limiting factor in wetlands (Vitousek and Howarth 1991) and is of particularly important factor in determining wild rice distribution and productivity (Keenan and Lee 1988, Lee 1986, Sims et al. 2012a). Similar to the findings of Meyerson et al. (1999) the nitrogen in porewater (Table 2) and sediment (Table 3 and Table 4) showed cattail dominance resulted in a net depletion of this element. In contrast, Angeloni et al (2006) showed cattail invasion resulted in a significant increase in nitrate and ammonium, and contributed it to the change seen in the denitrifer communities. Geddes et al (2014) showed an increase in denitrification but Lishawa et al. (2014) did not. The decrease of N (ext. NO_3, NH_3, avail N) may also be correlated to rhizosphere oxygenation by the invasive cattail which increases its availability and absorption (Morris and Dacey 1984, Lee 1978). Perhaps, the contradictory results can be explained by considering the impact time since invasion, which can influence nutrient dynamics (Mitchell et al. 2011). The study by Geddes et al. (2014) illustrated that an increased residence time of the invader resulted in higher organic matter, nitrate and ammonium and decrease denitrification.

Nitrogen was also influenced by removal, showing a lower concentration in the sediment in the treatment site for 2015 (Table 4). A similar trend of ammonium was reported by Meyerson et al. (1999). They associated the depletion in *Typha* dominated site to the invaders ability to control

soil nutrients through significantly higher aboveground biomass and that concentrations required three years to recover to pre-invasion conditions.

Several studies have shown the importance of phosphorus in determining of wild rice and its productivity (Lee 1982, Lee 1983b, Lee and Stewart 1984). The cattail-invaded site experienced a significant depletion of phosphorus in the sediment (Table 3), which may be explained by the significant increase in pH resulting in increased availability; This is supported by results seen in plant tissue analysis (Figure 6). In 2017, sediment pH was not significantly different between the treatment and cattail dominated and phosphorus values were similar. Also, increased oxygenation of the rhizosphere by the cattails versus wild rice (Conlin and Crowder 1989) may have influenced phosphorus availability. Oxidized iron bonds with phosphorus forming a precipitate and reducing availability (Reddy and Delaune 2008). Phosphorus in the treatment site was not significantly different from natural stand, at three years post-removal. This result suggests treatment allowed pre-invasion phosphorus levels to re-establish, which may have contributed to the successful re-establishment of wild rice.

The cattail invaded site showed a significant increase in soil organic matter (Table 4) in 2017 characteristic of cattail invasion (Tuchman et al. 2009, Geddes et al. 2014). This is critically important because an increase in soil organic matter gives a competitive advantage to macrophytes that have evolved in high nutrient environments (Van Der Putten et al. 1997) and can shift the soil microbial community creating a feedback and legacy effect that supports invaders (Geddes et al. 2014, Keyport et al. 2018). Several studies have attributed large litter accumulation (Freyman 2008, Farrer and Goldberg 2008, Larkin 2012,) as a result of high primary productivity and slow decomposition (Mitchell et al. 2011). The removal of cattails

resulted in the re-establishment of pre-invasion levels of organic matter (Table 4). This contrasts with Geddes et al. (2014) and Keyport et al. (2018) who reported legacy effect within treatment sites due to accumulation of litter. Although we did not measure litter accumulation, Rat River Bay is a high energy system and likely experiences periodic export of litter. In addition, cattail stocks normal act a as barrier for litter movement their removal may have contributed to the return to pre-invasion conditions

In the cattail-invaded area, soil moisture was significantly lower in 2015 but higher in 2017 (Table 4). This trend seems to be the result of the drastic decrease in the soil moisture within the treatment site between 2015 to 2017. Soil moisture indicates pore size and water content in the sediment which influences microbial activity, thus impacting nutrient availability (Bai et al. 2004, Skopp et al. 1990), especially nitrogen (Sluetel et al. 2008) and carbon mineralization (Yoo et al. 2006). Discriminant analysis (Figure 8) identified soil moisture as an important variable that distinguished cattail, wild rice and treatment sites. Also, soil moisture was highly correlated to total carbon and nitrogen. The insignificant difference in soil moisture between treated and natural stand in both 2015 and 2017 and the decrease in soil moisture within the treatment site suggests the influence of cattail dominance with a one-year legacy effect as the environment shift backs to pre-invasion conditions and wild rice dominance.

Cattails also appears to affect cycling of extractable anions and cations (Table 4), since their dominance resulted in elevated concentrations of Ca, Mg, and chloride in the sediment and decreases in the porewater (Table 2). Previous studies have shown that sediment nutrient concentrations can be altered by the aquatic plant and their changes are species specific (Moore

et al. 1995, Meyerson et al. 1999, Lee and McNaughton 2004). Possible mechanism for these changes is the variation in rhizosphere oxygenation (Wright and Otte 1999, Stoltz and Greger 2002) and nutrient uptake (Bai et al. 2012, Meyerson et al. 1999). The calcium decreases in the porewater of the cattail dominated site, may have been due to a higher demand for calcium that placed an increased demand on its release from the sediment. Also, Jorgenson (2013) reported the high Ca concentration in porewater of a wild rice versus a cattail dominated site and attributed the difference to the decrease in demand by wild rice.

The elevated potassium in cattail dominated site (Table 4), suggests possible leaching from litter, as potassium is readily released during decomposition (Boyd 1970, Sain 1984, Lee 1986, Thompson et al. 2009). The removal of cattails coincided with pre-invasion potentially indicating the loss of cattail control on soil potassium cycles comparable to Thompson et al. (2009) who also attributed elevated potassium to leaching under cattail dominance.

Metals in sediment were also affected by changes in dominant vegetation (Table 4) possibly caused by changes to the rhizosphere. Levels of Zn and Cu were higher in the cattail dominated site in 2015 but not 2017. Mn was significantly higher in the wild rice site in 2017 than either the treated or cattail site. Variations in nutrient uptake, oxygen release from the rhizomes of the cattails and variations in pH.

In terms of manganese, Gotoh and Patrick (1972) reported that once pH drops below 6 redox has little effect on manganese solubility. Therefore, the decrease in total and extractable in the cattail dominated area, may be due to the significantly higher aerobic cattail stand and increased

concentration in plant tissue. Additionally, there was no significant difference in manganese in 2015 but pH was much closer to 6.

Redox was measured in 2017 and results showed a significantly higher level in cattails (table 4), presumably due to variation in rhizosphere oxygenation. Rhizosphere oxygenation is species specific (Brix 1993, Wright and Otte 1999, Stoltz and Greger 2002,) and wild rice and cattails differ greatly in their root structure. Cattails form colonies with a massive underground network of rhizomes and connected stocks that allow for increased oxygen transport (Jordan and Whigman 1988, Brix et al.1992). The proceeding chapter will examine in detail the ability of cattails to influence wild rice.

1.4.3 Conclusion and Management Implication

The first objective was to evaluate mechanical removal of cattails as an effective method for wild rice re-establishment. In this study we had no re-invasion three years post treatment, and wild rice was able to voluntarily re-establish and retain dominance. The community-level study of cattail dominance and elimination indicates that cattails have a substantial effect on the biogeochemistry (objective 2) and their dominance altered primary productivity, nutrient cycling, porewater chemistry, redox potential and pH. However, the impact seems to be reversible when cattails are removed.

As a management strategy, some considerations must be contemplated during implementation, such as a repeated treatment. This would likely be required due to the cattail's superior ability to outcompete wild rice. However, my results show that removal could occur on a three-year

rotation. Another consideration is the large-scale collection of cattail biomass being present after cutting. However, several studies have investigated the use of cattail biomass as an alternative energy source which could be an effective way to offset the cost of removal. This study has implications for the management of cattail invasion in fresh water wetlands, but equally important is the successful re-establishment of wild rice. The successful and sustained wild rice stand seemed to negate any concern of legacy effects. While the passive re-establishment of wild rice due to removal is conceptually encouraging it remains unclear how cattail invasion impacts seed bank viability and germination.

Chapter 2
Cattail (*Typha angustifolia L.*) Mediated Alteration of Microenvironment with Impacts on the Germination of Wild Rice (*Zizania palustris* L.)

2.1 Introduction

Invasive species are one of the leading causes of biodiversity loss (Wilcove et al. 1998, Mack et al. 2000), and are a major threat to ecosystem services (Parker et al. 1999 Mack et al. 2000). Invasive wetland plants are robust, adaptable and prolific, allowing them to easily out compete natives (Vitousek 1990). They alter productivity, nutrient cycling and geomorphology to an extant in which natives no longer survive (Mack et al. 2000, Ehrenfeld 2003, Liao et al. 2008, Vaccaro et al. 2009). These changes and the change in dominate plant species can impact the native plants seed bank in terms of viability, dormancy and germination (Xiong and Nilsson 1999*). Typha angustifolia*, like many invasive plants creates extensive dense monospecific stands (Vaccoro 2005). Their water depth tolerance as much as 1.5m (Tornberg et al. 1994) and their prolific nature (Linde et al. 1976) devastates local species including the highly valued northern wild rice (*Zizannia palustris*).

Wild rice is an annual plant so its viability during dormancy and successful induction of germination is critical for its survival and successful re-establishment (Moyle 1944, Jenks 1989). *Z. palustris* seed enters a primary dormancy period that begins after separation from the parent plant, called the after-ripening period. This period occurs in cold, anoxic sediment for a minimum of three months (Simpson 1966, Atkins 1986). The maximum length of viability is not well documented however; Oekle et al. (1982) found successful germination of wild rice after six years of complete crop failure. The length of the dormancy and the induction of germination has

been suggested to be the result of seed coat impermeability as germination dramatically increases when seeds were scraped above the embryo (Simpson 1966, Cardwell 1977). Permeability inducing germination or an extended dormancy is believed to be cued from some combination of environmental factors such as temperature, oxygen and light (Cardwell 1977, Atkins 1986). Germination will not occur at a temperature below 4°C (Simpson 1966, Atkins 1986). Extended dormancy allows seeds to remain viable during unfavourable conditions and only germinate when the chance of survival increases (Gutek 1975, Atkins 1986). Little is understood about conditions required for germination or what induces an extended dormancy, but it serves an import role in the ecology of wild rice.

Typha, like many emergent macrophytes alleviates the stress of an anoxic environment through aerencyhmous lacunae tissue that allow the movement of oxygen from exposed leaves to submerged roots (Armstrong 1972). Excess oxygen is released into the environment creating an oxygenated micro-zone, the extent of which is dependent on the capacity of the plant to transport oxygen, the demand of the submerged organs, and the reducing potential of the sediment (Armstrong 1992). Although, this adaptation is common in emergent macrophytes, *T. angustifolia* has an internal pressurization and connected flow through that greatly increases the flow of oxygen (Brix et al. 1992, Tornbjerg et al. 1994). This allows them to grow in deeper water (Tornbjerg et al. 1994) and increases the radial oxygen loss from the rhizosphere (Armstrong and Armstrong 1990). Several studies report radial oxygen loss to an extent emergent aereanchymous plants facilitate neighbouring non-aerencyhmous (Callaway and King 1996). There are conflicting results in the facilitation or impact of oxygenation by macrophytes as several studies have reported macrophytes with significant transport but no measurable change

in the oxygen concentration in the root zone (Morris and Dacey 1984, Brix 1988, Bedford et al. 1991). This may be due to the oxidization of elements in the root zone and by heterotrophic and autotrophic oxidation by bacteria, as well as diffusive resupply across oxygenated zone (Reddy and Delaune 2008).

Many wetland macrophytes including *Zizania texana* (Power and Fonteyn 1995) and *Oryza sativa* (Magneshi and Perata 2008) experience an inverse relationship between germination and dissolved oxygen (Svare 1960, Leck 1996, Lorenzen, 2000, Wijte and Gallager, 2000). It is thought to be an adaptation due to flooded conditions (Leck 1996) but this is not always the case, even for obligate wetland plants (Fraser et al. 2014). There is conflicting evidence regarding the role of dissolved oxygen concentration in the dormancy and germination of *Z. palustris* (Atkins1986). Svare (1960) reported increased germination with decreased dissolved oxygen, but others reported high germination without deoxygenation (Simpson 1966, Campiranon and Koukkari 1977). These conflicting results may be due to the difference in dormancy state and the use of scraped seeds as in Campiranon and Koukkari (1977).

Litter is also an important factor in determining community structure (Xiong and Nilsson 1999) because of its influence in affecting the physical and chemical environment (Facelli and Picket 1991). It creates a physical barrier preventing seed germination, as well as a microclimate that is considerably different from pre-invasion conditions (Xiong and Nilsson 1999). Secondly, the accumulation of litter consumes available oxygen from consumption during microbial decomposition. Thirdly, leachate from *Typha* litter has been reported to have allelopathic properties preventing the growth or germination of native plants (Bonasera et al. 1979, Jarchow

and Cook 2009, Szabo et al. 2018,). Litter from invasive *Typha* was to be the main driver of ecosystem change and mechanism for dominance.

The results of the study, in addition to those discussed in Chapter 1, show that invasive cattails dramatically alter their microenvironment and that wild rice was able to voluntarily re-establish within treatment zone. The objectives of this study were to i) examine the mechanism in which cattails alter their micro-environment and to ii) to determine if the presence of cattails impacted the germination of wild rice.

2.2 Materials and Methods

2.2.1 Mesocosms

During the summer of 2017, sediment samples collected from Rat River Bay (48°37'19.80"N, 92°39'14.31"W) was homogenized and divided equally into twelve 42 L plastic buckets and placed in a larger mesocosm (Figure 11). The mesocosm was set up with a continuous flow of river water and a 2-inch stand pipe to sustain a water depth of 1.2 meters. A cattail treatment (n=6) was planted with *T. angustifolia* collected from Rat River Bay at mean density found in the field site. Unplanted treatment was kept plant free. All buckets were fitted with two monitoring wells (Figure 10). The wells were constructed using a PVC pipe with a diameter of 8 cm, the bottom 5 cm were perforated and covered in 1000 μm Nitex mesh. Each monitoring well was capped at the top and bottom; the top cap only removed during sampling or purging events. After planting there was an acclimation period of two weeks to allow the cattails to establish.

Sediment and river water were tested prior to start date and on Day 1, 2, 4, 7, 9, 10, 11 after stabilization the monitoring wells were tested weekly. All monitoring wells were purged until dry and sampled within 24 hours. Parameters measured included pH, dissolved oxygen (DO), temperature, conductivity and redox. Monthly samples were collected from representative monitoring wells for anions, nutrients and metals analysis.

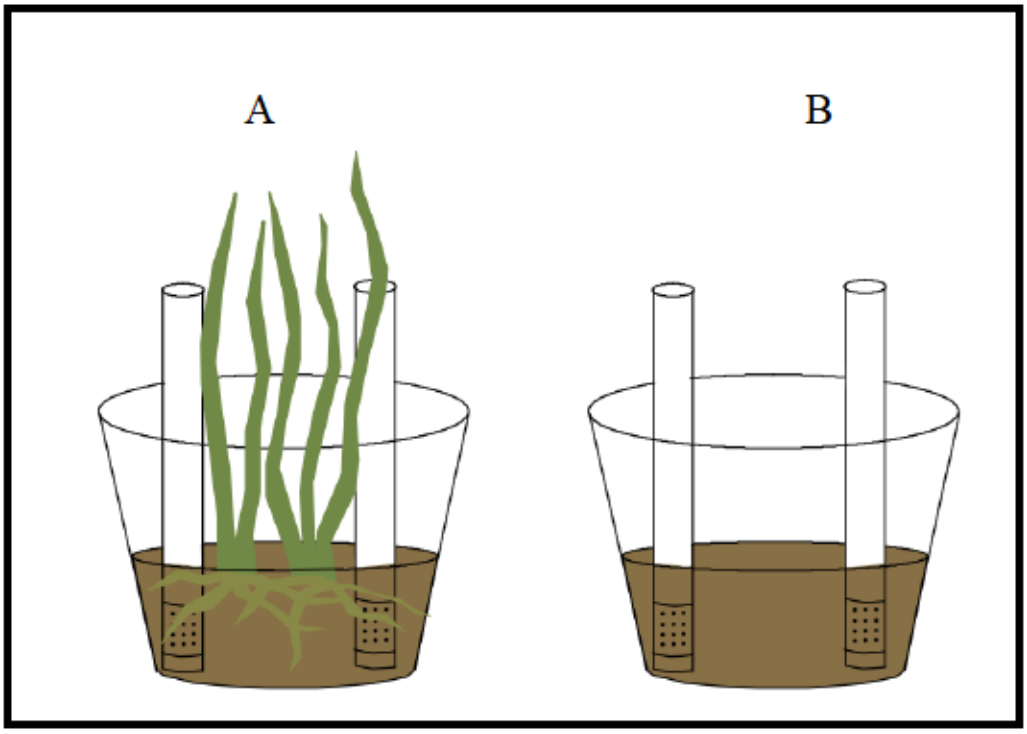

Figure 10. Mesocosm treatment setup A) *Typha* planted B) unplanted buckets within mesocosm setup

Figure 11. Mesocosm set up with cattail planted (n=6) and unplanted (n=6) treatments with reservoir tank

2.2.2 Wild rice Germination Trials on Cattial Litter

Wild rice seeds were collected from Rat River bay in September of 2015; and were afterrippened in a cold, damp environment at a constant temperature of 4° C. After six months they were removed from storage, dehulled and sterilized with 10% hydrogen peroxide for 30min, to reduce the risk of mold. The seeds were immediately placed in Perti dishes with one of four treatments: fresh cattail leaves, cattail roots, cattail rhizomes and distilled deionized water (DDW). Each treatment had three replicates with six seeds each. The experiment was conducted in a growth chamber, which had a daily schedule of 16-hour photoperiod at 25 °C followed by 8 hours dark at 12 °C. Water (DDW) level was maintained daily, and a count of germination taken every 48 hours.

2.2.2.2 Dissolved Oxygen Germination

Wild rice seed from Rat River bay was collected in 2016; they were treated as above and placed in beakers in of three treatments: low oxygen, high oxygen and DDW. Low oxygen was achieved by placing all replicates in a GLOVE bag that was filled with nitrogen gas and replaced daily. High oxygen treatment was achieved by continually bubbling ambient air in each replicate. Each treatment had 9 dishes and 10 seeds in each dish with a total of 90 seeds per treatment. All treatments were place in a growth chamber with a daily 16-hour photoperiod at 20°C followed by 8-hours dark at 12 °C. Water level was maintained daily, and a count of germination occurred every 48 hrs.

2.2.3 Data Analysis

A one-way ANOVA was used to analyze difference in germination with LSD post hoc analysis to determine significant difference between treatments. I utilized a repeated-measures analysis of variance (ANOVA) to compare variables between treatments throughout the mesocosm trial, Wilks λ was used as the separating statistic with the probability for inclusion of variables set at $P< 0.05$. All statistics were run using SPSS version 25.

2.3 Results

2.3.1 Mesocosm

The mesocosm ran from August 12th to October 4th 2017 and the perforated monitoring wells provided access to porewater. Of the porewater chemical variables measured throughout the experiment dissolved oxygen (P=0.031), pH (P=0.000), conductivity (P=0.000), Eh (P=0.033), and temperature (P=0.000) changed significantly over time. (Figure 12). Dissolved oxygen in the

monitoring wells stayed between 0 and 2 mg/L while the water column varied between 6 and 12 mg/L. There is a very slight difference in mV between the cattail treatment and unplanted control, also the cattail treatment was slightly oxidized and the difference seemed to increase as temperature decreases.

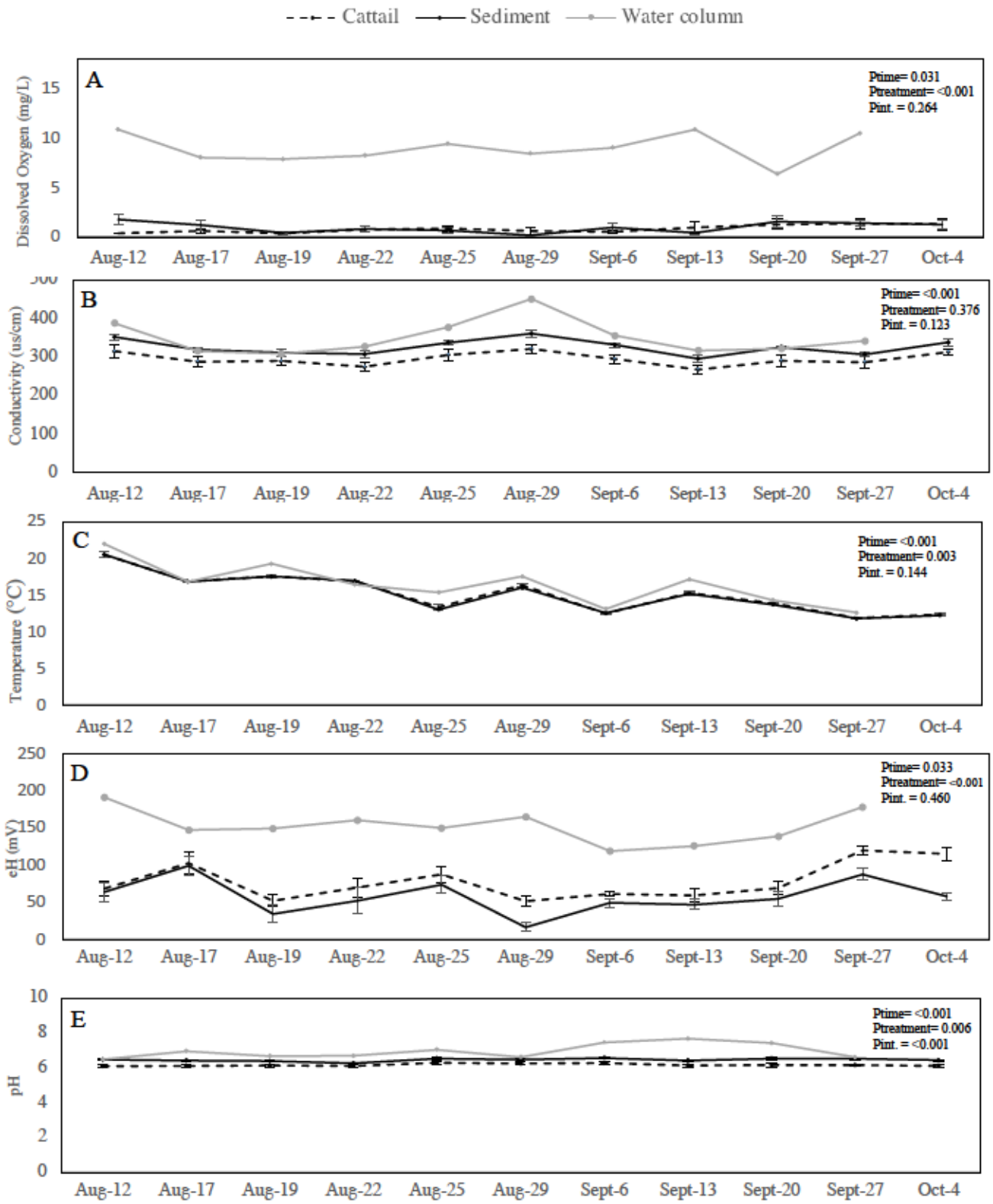

Figure 12. Average ±Standard error of porewater results for planted and unplanted treatments for A) conductivity B) Dissolved Oxygen C) Temperature D) eH throughout the duration of the mesocosm experiment.

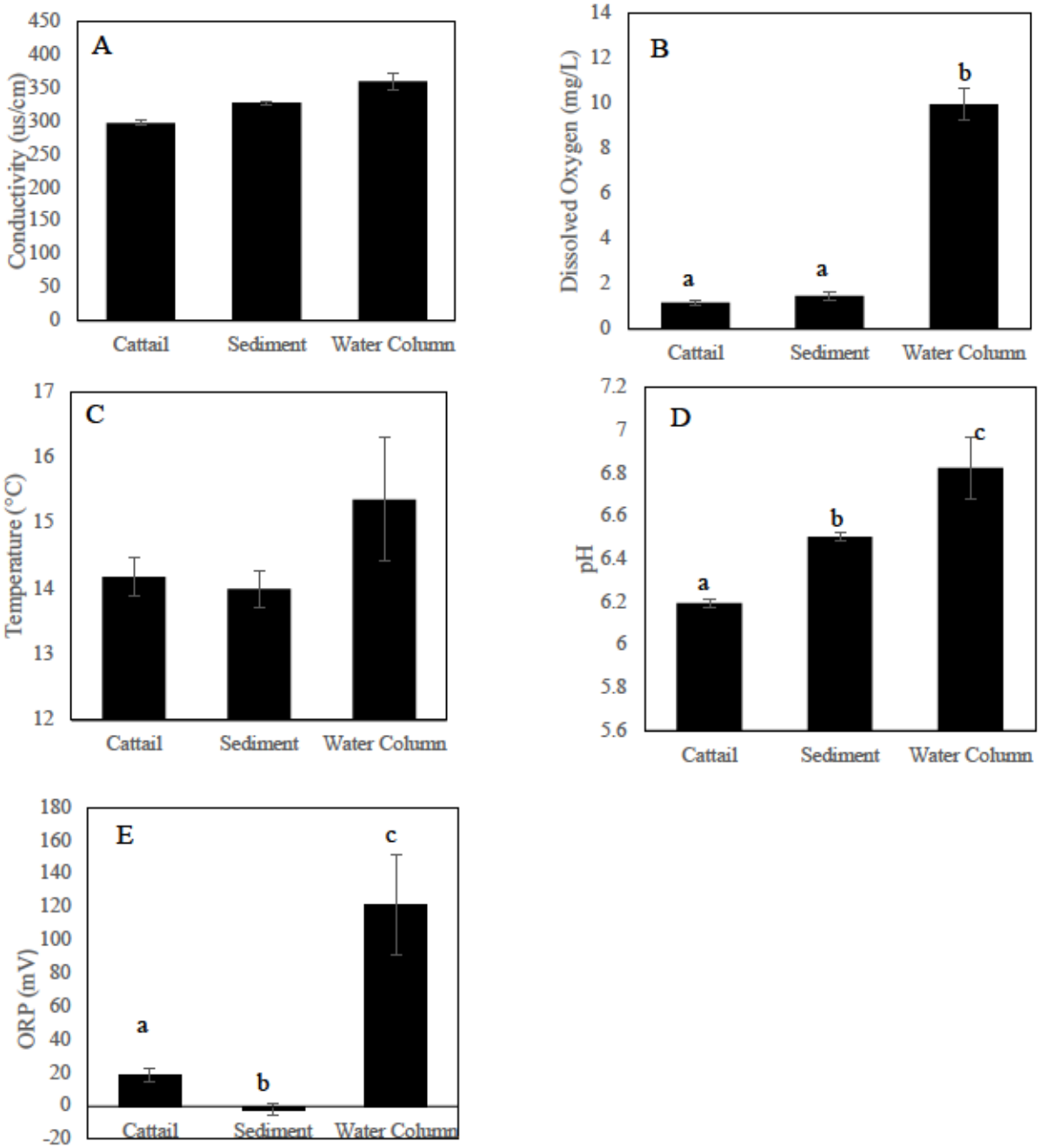

Figure 13.Average porewater values taken throughout the entire experiment error bard represent standard error A) conductivity B) Dissolved oxygen C) Temperature D) pH E) ORP

The differences between values averaged for the entire of the mesocosm are shown in Figure 13. The average dissolved oxygen concentration in cattail treatment was 1.2 mg/L compared to the sediment treatment of 1.5 mg/L but both were significantly lower than the results seen in water column ($P_{CT\ vs\ WC} < 0.001$; $P_{SED\ VS\ WR} < 0.001$). The porewater pH was highest in water column and lowest in cattail planted treatment ($P_{CT\ vs\ SED} < 0.001$; $P_{CT\ vs\ WC} < 0.001$; $P_{SED\ vs\ WC} < 0.001$). Oxygen reduction potential was the most reduced in the unplanted sediment treatment and was significantly different from the cattail planted treatment ($P_{CT\ VS\ SED} < 0.001$; $P_{CT\ VS\ WC} < 0.001$).

2.3.2 Wild rice Germination Trials on Cattial Litter

The germination of seeds of wild rice subjected to cattail leaf litter did not differ significantly from control seed germination ($P_{leaves\ vs\ control} = 0.650$) (Figure 14). Wild rice germination on cattail root litter ($P_{root\ vs\ DDW} = 0.011$) and cattail rhizomes ($P_{rhizome\ vs\ DDW} = 0.011$) were significantly higher than seeds in DDW (Figure 15). In addition, both the rhizomes treatment and root treatment were significantly higher than leaf litter ($P_{roots\ vs\ leaves} = 0.022$) and rhizome litter ($P_{rhizomes\ vs\ leaves} = 0.022$) (Figure 14).

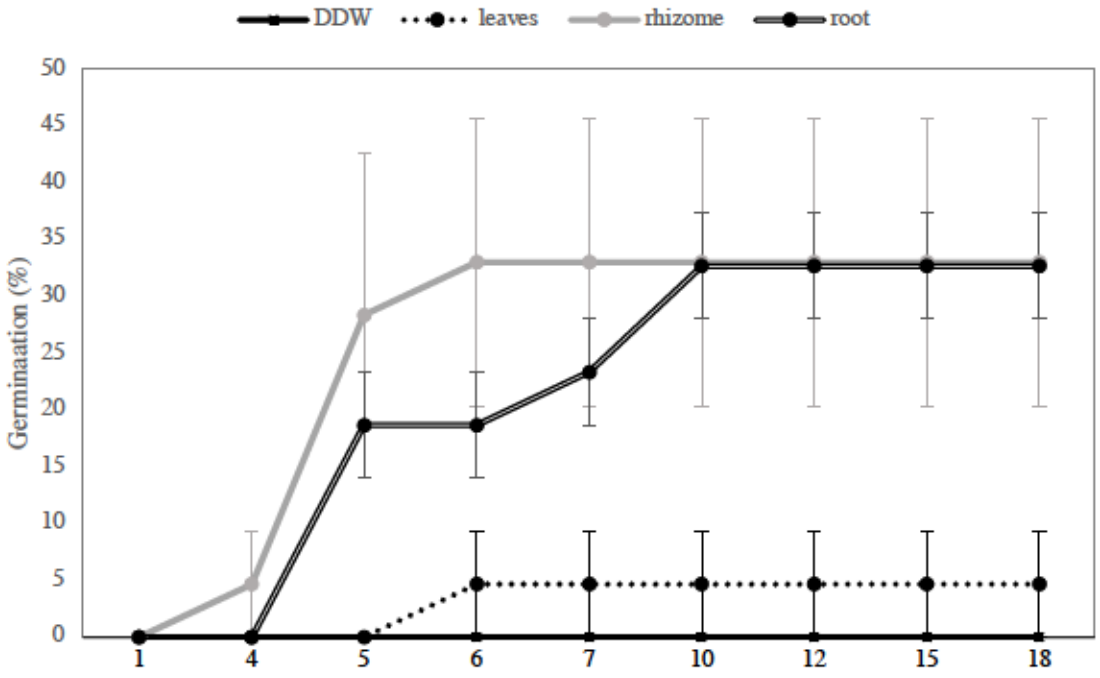

Figure 14. Mean germination of wild rice seeds in cattails roots, rhizomes and leaves. Error bars represent standard error

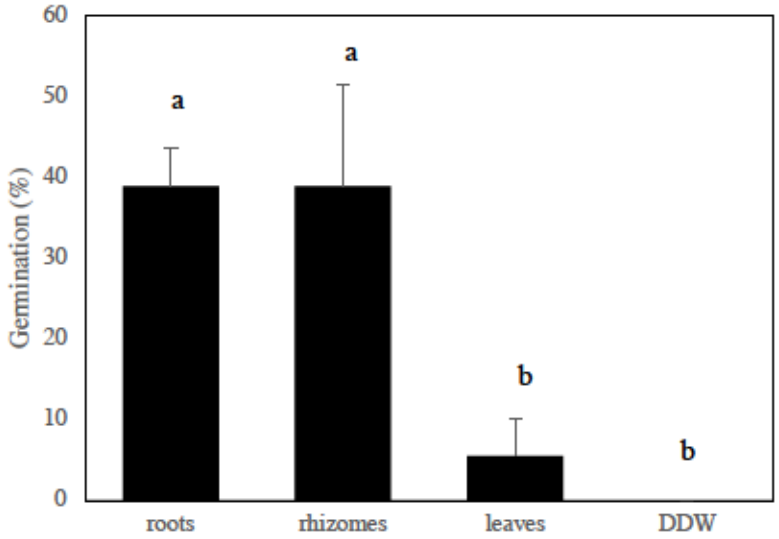

Figure 15. Percent germination of wild rice in DDW with cattail roots, rhizomes and leaves. Error bars represent standard error and different letters indicate significant differences between treatments according to LSD post hoc test.

2.3.3 Dissolved Oxygen Germination Trial

Subjecting the wild rice seeds to differing levels of oxygen resulted in significantly different rates of germination. Under low O2 wild rice germinated seven days earlier (Figure 17) with significantly higher total germination ($P_{\text{low vs high}} = <0.001$; Figure 17). The highly oxygenated treatment and the DDW treatment both experienced a seven-day delay in germination (Figure 16) with no difference between treatments ($P_{\text{high vs ddw}} = 0.420$) (Figure 16)

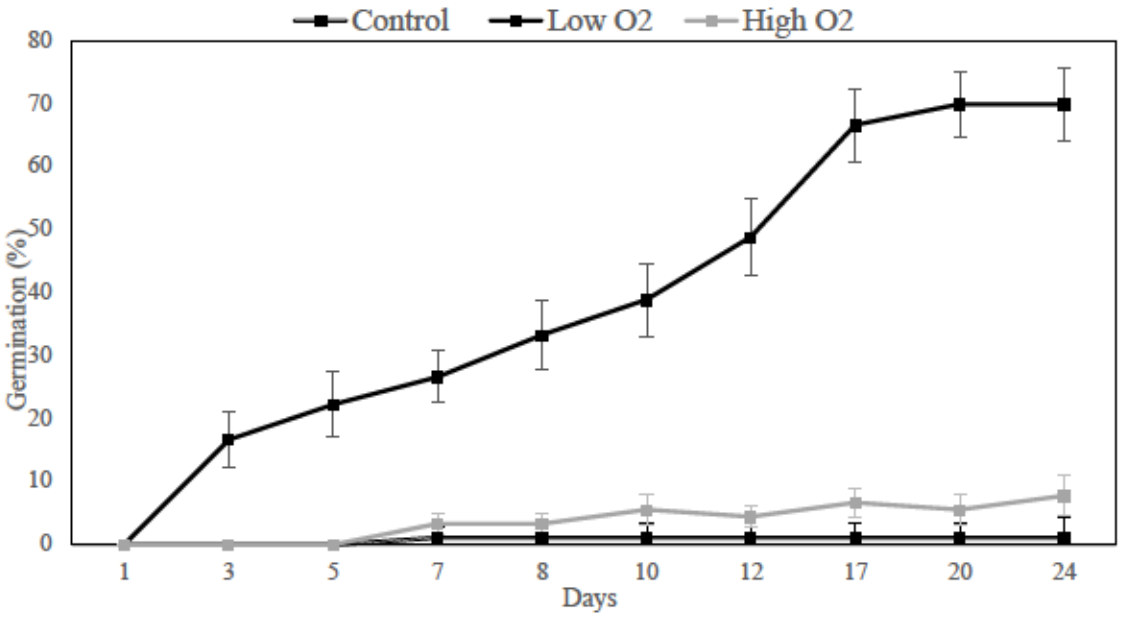

Figure 16. Mean percent germination of wild rice in Low O2 treatment, high O2 treatment and DDW. Error bars are standard error

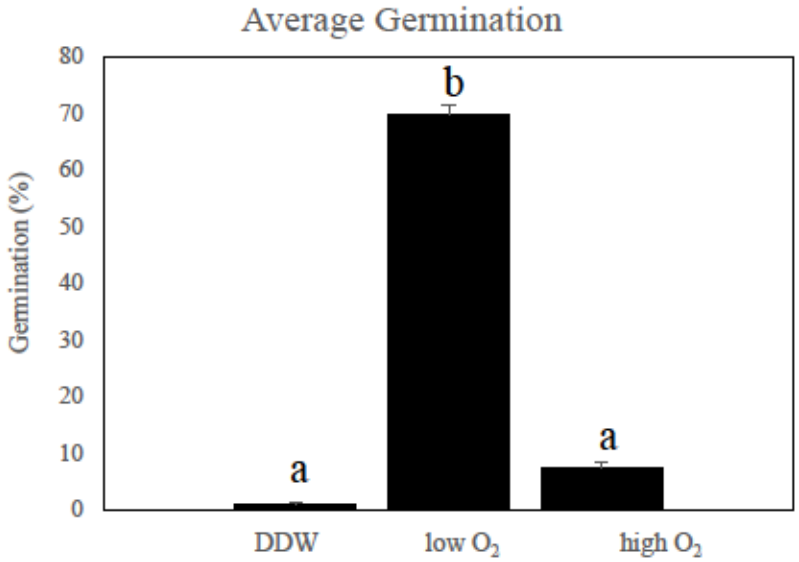

Figure 17. Mean percent germination of wild rice in ambient air, high O2 and low O2. Error bars represent standard deviation and bars sharing the same letters are not significantly different according to Tukey's LSD post hoc test

2.4 Discussion

2.4.1 Mesocosm

The objective of the mesocosm experiment was to examine *Typha angustifolia's* ability to alter its root zone to an extent to which there was a detectable change in porewater chemistry. The results indicated that over the span of our study the large, highly aerenchymous invasive *T. angustifolia* significantly altered conductivity, pH and ORP but no difference was detected for dissolved oxygen and temperature. Also, the within subject analysis of dissolved oxygen, pH, conductivity and Eh all changed significantly with duration of the experiment. These results are consistent with others showing that increased residency time of T. angustifolia results in an increased impact.

Typha angustifolia have a greater ability to transport oxygen into their submerged organs, allowing them to invade deeper waters (Tornberg et al. 1994). However, like results, several studies on other macrophytes with significant transport but no measurable change in the oxygen concentration in the root zone (Bedford et al. 1991, Morris and Dacey 1984, Brix 1988). This may be due to the oxidization of elements in the root zone and by heterotrophic and autotrophic oxidation by bacteria, as well as diffusive resupply across oxygenated zone (Reddy and Delaune 2008). Jeperson et al. (1998) reported that the high oxygen demand in the sediment masked the effect of root oxygen release. The DOC in porewater reported here is comparable to those reported Callaway and King (1996), who report a significant difference in net oxygen loss but only when the experiment was performed at a temperature below12°C, attributed these results to the decrease in respiration (oxygen consumption) that would occur at the lower temperatures. A similar trend was also seen in *Spartina alterniflora* (Howes and Teal 1994). As the mesocosm was outdoors, temperature control was not possible but the trend in eH in (Figure 13) suggest a net difference was increasing during the latter portion of the growing season and therefore the potential for complimentary results existed if the experiment was extended into November. This result would be critical, as it's suggesting net oxygenation would then occur while wild rice seed is in the sediment.

Different techniques used to measure rhizosphere oxygenation are often blamed in the contradictory results. Several studies use a method involving plant cuttings in an anoxic solution to measure net oxygen change, which doesn't consider respiration, sediment oxygen demand or reduction state or the sediment (Sorrell and Armstrong, 1994). While the methods in this study was chosen due to objectives of gaining a better understanding of our field site and therefore

using sediment from the study site and whole plants includes all elements factoring in cattails ability to impact neighbouring species (Bedford et al. 1991).

The significant decrease in pH in the planted treatment suggests oxygenation of the rhizosphere which decreases metal availability as they are transformed to their oxygenated state (Reddy and Delaune 2008). The decrease of pH under *Typha* dominance is similar to those reported by Geddes et al. (2014). Redox potential (ORP) was significantly less reduced in the *Typha* planted treatment than the unplanted treatment. Redox potential (ORP) did not follow the same trend as dissolved oxygen and was significantly different between planted and unplanted treatments. Oxygen reduction potential is commonly used to infer oxygenation but redox is influenced by many other factors and provides only a qualitative indication of oxygen. All site redox values were below 300 mV which indicate reducing conditions both within the range of iron reduction (Reddy, 2000)

2.4.2 Wild Rice Germination Trials on Cattail Litter

The litter germination trial experienced drastically different germination rate as were observed in the germination trial (Figure 18). Rhizome and root treatments experienced significantly higher germination when compared to leaf and DDW treatment. As germination in all three cattail plant tissue treatments (roots, rhizomes and leaves) was higher than the DDW treatment, it suggests cattail tissue does not inhibit the germination of wild rice and that allelopathy from litter would not play a role in wild rice seed bank dynamics. A similar trend reported by Bonasera et al. (1979) who showed that *Typha* root and rhizome litter had no inhibitory effects on the germination of native species. Though more research is required to say whether invasive cattail has an allelochemical impact on the germination of wild rice, as there are several other studies

indicating biologically active substances in cattail tissue that affect the germination of neighbouring species (Gallardo et al. 1998, Gallardo et al. 2002, Jarchow and Cook 2009). The higher germination in the rhizome and root treatments could be explained by the decomposition that occurred during the trial consuming oxygen and potentially creating the preferred anaerobic environment. The leaves did not differ significantly from the DDW treatment and may be due to the slow breakdown of leave tissue due to the cuticle (Zukswert and Prescott 2017).

2.4.3 Dissolved Oxygen Germination Trial

We found the highest germination of wild rice seed in the low oxygen treatment in comparison to control and high O_2 (Figure 15) similar to results reported by Svare (1960). Several marsh species are also reported to show an inverse relationship between oxygen concentration and germination including *Scirpus juncoides* L. (Pons and Schroder 1986), *Scirpus lacustris* (Clevering 1995), *Typha latifolia* L. (Bonnewell et al.1983), *Oryza Sativa* L. (Miro and Ismail, 2013) and *Pontederia cordata* L. (Leck 1989). For obligate wetland plants, low oxygen concentration is a characteristic of the environment in which seed would afterrippen and germinate but it is important to note this inverse relationship is not the norm. It is actually more common to see oxygen depletion impeding germination of wetland plants (Fraser et al. 2014).

The mechanism required for germination in an anaerobic environment depends upon alcoholic fermentation by the enzyme alcohol dehydrogenase (ADH), which has been reported in wild rice seeds by Campiraninn and Koukkari (1977). Which is an anaerobic pathway for generating the energy needed for growth (ATP's), that is highly specific to ethanol as a substrate (Mitsch and Gosselink 2000). Therefore, seeds must also have some mechanism to tolerate the ethanol production, usually through venting to the external environment (Bertani et al. 1980). Rumpho

and Kennedy (1981) reported that ethanol produced in the anaerobic germination of *Echinochloa crus-galli* L. was 90 times greater than in the ambient air treatment with 98% of it located in the external media.

The dissolved oxygen germination trial show minimal germination in aerated and DDW treatment contradictory to results reported by Atkins (1986) and Campiranon and Koukkari (1977). This contradictory result may be due to the multiple mechanisms that contribute to dormancy such as the mechanical resistance of water and gasses by the pericarp, the water-soluble inhibitors present in the hull and pericarp, plus gibberellic acid concentrations are lowest in freshly harvested seeds (Barton and Crocker 1953, Simpson 1966, Cardwell 1978). Thus, differences in the how the seeds were stored as well as the manual removal of dormancy in the Campiranon and Koukkari (1977) study could explain the high rate of germination regardless of dissolved oxygen concentration. The lack of germination in the high oxygen and DDW treatments could also be a result of a secondary dormancy supported by Simpson et al. (1966) who reported an increased dormancy under high oxygen tensions. In addition, the importance of dissolved oxygen was also hypothesised by Atkins (1986), who could not explain trends seen in the germination of wild rice by temperature alone. The Cardwell (1978) study also noted the decreased impact of manually breaking the seed coat after an extended after-ripening and postulated that the introduction of an extended or secondary dormancy was due to the pericarp impermeability to gases.

Timing of germination was affected by O_2 concentration as the control and bubbling of ambient air was delayed for 7 days compared to the low O_2 treatment. Such as an anaerobic environment

would give wild rice a competitive advantage with a higher success rate in addition to early emergence of seedling from the sediment (Miller 1987, Stockey and Hunt 1994).

2.4.3 Conclusion

The results of this study show that invasive *Typha angustifolia* can alter its environment, which may be impacting the germination of native wild rice. The mesocosm demonstrated that cattails in the buckets lowered pH and increased oxidation reduction potential versus buckets with no cattails. The germination experiments revealed that wild rice germination was significantly higher in the cattail rhizome and root treatments versus the cattail leaves. Germination was also significantly higher in the anaerobic treatment versus the other treatments.

Summary and General Conclusions

The invasion of *T. angustifolia*, like several other invasive wetland macrophytes, creates dense monospecific stands that completely eradicates native species. Management techniques of this invader are increasingly important as the continued expansion of *Typha* into vulnerable wetlands results in the loss of biodiversity and ecosystem services (Ehrenfeld 2003, Lishawa et al. 2010).

This thesis showed that successful re-establishment of wild rice could occur if cattail removal occureed as there was negligible regrowth. Cattails did have an impact on the nutrient environment causing depletion of macro and micronutrients with nitrogen depletion being particularly noticeable. This was thought to have a long-term impact on the sustainability of the re-established wild rice. Germination of wild rice was stimulated by conditions causing a more reduced environment. The suggested mechanism was the release of oxygen from cattail rhizomes which resulted in a loss of wild rice germination. Our hypothesis that removal of cattails could result in wild rice re-establishment was correct but longer-term studies are needed to determine if the wild rice would produce sustainable populations given the alteration of the sediment by cattails.

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Appendices

Appendix A Data Tables

Table A.1 Rat River Bay Stem density biomass and weight per plant of Cattail dominated area (CT), natural wild rice stand (WR) and a treated wild rice area (TWR)

Parameters	Data test (P≥ 0.05) SQUAREROOT TRANSFORMED								ANOVA (P≤ 0.05)		
	Normality of Treatment (P≥0.05)			Normality of Year (P≥0.05)			Equality of variance(P≥0.05)				
	Facto	Result	P	Facto	Result	P		P		P	F
Number of plants per meter2	WR	Pass	0.470	2015	fail	0.006	Fail	0.002	Treat	0.000	24.597
	TWR	Pass	0.089	2017	pass	0.155			Year	0.737	0.114
	CT	Pass	0.581						Inter	0.078	2.664
Dry weight m2 …biomass?	WR	Pass	0.107	2015	Fail	0.012	Fail	0.009	~~Treat~~	0.000	57.256
	TWR	Pass	0.246	2017	Fail	0.012			~~Year~~	0.152	2.110
	CT	Pass	0.717						~~Inter~~	0.026	3.912
Weight per plant	WR	Pass	0.716	2015	Fail	0.018	Fail	0.033	Treat	0.000	110.637
	TWR	Fail	0.033	2017	Fail	0.002			Year	0.101	2.778
	CT	Pass	0.974						Inter	0.006	5.641

Table A.2. Plant Tissue results IN UG/G of One-way ANOVA using factor of treatment site (cattail dominated: CT, wild rice (WR) and Treated site (TWR). Significant difference was determined using LSD post hoc analysis.

One-Way ANOVA Plant tissue µg/g
(Treated, Wild rice and Treated)
2015 and 2017
Factors: Treatment

Parameters	Data test (P≥ 0.05)					ANOVA (P≤ 0.05)				Treatments	LSD p-value
	Treat	Normality of Treatment (P≥0.05)		Equality of variance(P≥0.05)							
		Result	P	Result	P	F	P	Sign	df		
Aluminum	WR	Fail	0.000	Fail	0.001	9.968	0.000	Sign	2	WR vs TWR	0.243
	TWR	Fail	0.000							WR vs CT	0.000*
	CT	Pass	0.546							TWR vs CT	0.002*
Barium	WR	Pass	0.155	Pass	0.336	3.209	0.047	Sign	2	WR vs TWR	0.708
	TWR	Pass	0.401							WR vs CT	0.019*
	CT	Pass	0.346							TWR vs CT	0.050*
Calcium	WR	Pass	0.627		0.000	5.918	0.004	Sign	2	WR vs TWR	0.733
	TWR	Fail	0.015							WR vs CT	0.005*
	CT	Pass	0.140							TWR vs CT	0.002*
Chromium	WR	Fail	0.000	Pass	0.113	0.402	0.670	Not	2	WR vs TWR	0.440
	TWR	Fail	0.000							WR vs CT	0.955
	CT	Fail	0.000							TWR vs CT	0.444
Copper	WR	Fail	0.030	Pass	0.051	0.444	0.643	Not		WR vs TWR	0.701
	TWR	Fail	0.038							WR vs CT	0.550
	CT	Pass	0.863							TWR vs CT	0.351
Iron	WR	Fail	0.000	Fail	0.000	17.726	0.000	Sign	2	WR vs TWR	0.266
	TWR	Fail	0.000							WR vs CT	0.000*
	CT	Fail	0.002							TWR vs CT	0.000*
Potassium	WR	Pass	0.768	Pass	0.591	18.072	0.000	Sign	2	WR vs TWR	0.010*
	TWR	Pass	0.145							WR vs CT	0.000*
	CT	Pass	0.068							TWR vs CT	0.001*
Magnesium	WR	Pass	0.504	Fail	0.030	1.374	0.261	Not	2	WR vs TWR	0.103
	TWR	Pass	0.323							WR vs CT	0.519
	CT	Fail	0.014							TWR vs CT	0.376
Manganese	WR	Fail	0.001	Fail	0.000	33.048	0.000	Sign	2	WR vs TWR	0.931
	TWR	Pass	0.594							WR vs CT	0.000*
	CT	Pass	0.340							TWR vs CT	0.000*
Sodium	WR	Fail	0.000	Pass	0.156	9.478	0.000	Sign	2	WR vs TWR	0.006*
	TWR	Fail	0.048							WR vs CT	0.096
	CT	Pass	0.121							TWR vs CT	0.000*
Nickel	WR	Fail	0.000	Pass	0.166	0.839	0.437	Not	2	WR vs TWR	0.391
	TWR	Fail	0.000							WR vs CT	0.217
	CT	Fail	0.000							TWR vs CT	0.676
Phosphorus	WR	Pass	0.469	Fail	0.003	7.547	0.001	Sign	2	WR vs TWR	0.143
	TWR	Pass	0.278							WR vs CT	0.00*
	CT	Pass	0.256							TWR vs CT	0.019*
Sulfur	WR	Fail	0.000	Fail	0.002	8.891	0.000	Sign	2	WR vs TWR	0.142
	TWR	Fail	0.005							WR vs CT	0.000*
	CT	Pass	0.227							TWR vs CT	0.009*
Silicon	WR	Fail	0.003	Fail	0.001	9.793	0.000	Sign	2	WR vs TWR	0.715
	TWR	Fail	0.005							WR vs CT	0.00*
	CT	Pass	0.222							TWR vs CT	0.00*
Strontium	WR	Pass	0.779	Fail	0.022	0.890	0.416	Not	2	WR vs TWR	0.694
	TWR	Pass	0.125							WR vs CT	0.192

	CT	Pass	0.312							TWR vs CT	0.362
Titanium	WR	Fail	0.006	Fail	0.000	8.903	0.001	Sign	2	WR vs TWR	0.049*
	TWR	Fail	0.001							WR vs CT	0.000*
	CT	Fail	0.000							TWR vs CT	0.044*
Zinc	WR	Pass	0.990	Pass	0.381	3.862	0.026	Sign	2	WR vs TWR	0.019*
	TWR	Fail	0.001							WR vs CT	0.872
	CT	Pass	0.282							TWR vs CT	0.020*
Total Carbon %	WR	Pass	0.508	Fail	0.003	247.76	0.000	Sign	2	WR vs TWR	0.038*
	TWR	Pass	0.047							WR vs CT	0.000*
	CT	Pass	0.344							TWR vs CT	0.000*
Total N	WR	Fail	0.001	Pass	0.097	8.470	0.001	Sign	2	WR vs TWR	0.003*
	TWR	Fail	0.027							WR vs CT	0.000*
	CT	Pass	0.464							TWR vs CT	0.231
C:N	WR	Fail	0.000	Pass	0.276	5.516	0.006	Sign	2	WR vs TWR	0.163
	TWR	Fail	0.000							WR vs CT	0.002
	CT	Pass	0.051							TWR vs CT	0.058
C:P	WR	Fail	0.000	Pass	0.192	8.740	0.000	Sign	2	WR vs TWR	0.726
	TWR	Fail	0.001							WR vs CT	0.000
	CT	Fail	0.011							TWR vs CT	0.001

Table A.2. Plant Tissue results in mg/m2 of Two-way ANOVA using factor of treatment site (cattail dominated: CT, wild rice: WR and Treated site: TWR) and year (2015 and 2017) Significant difference was determined using Post Hoc analysis

Plant tissue mg/g Two-Way ANOVA (Treated, Wild rice and Treated) Factors: Treated and Year, Log Transformed													
	Data test (P≥ 0.05)								ANOVA P≤ 0.05				
	Treat	Normality of Treatment (P≥0.05)		Normality Year (P≥0.05)			Equality of variance(P≥0.05)						
		R	P	Year	R	P	R	P		F	P		
Al	WR	Pass	0.291	2015	Pass	0.202	Pass	0.098	Treat	5.477	0.007	WR vs TWR	0.074
	TWR	Fail	0.013	2017	Pass	0.182			Year	4.284	0.043	WR vs CT	0.002
	CT	Pass	0.624						Inter	5.027	0.010	TWR vs CT	0.149
Ba	WR	Pass	0.697	2015	Pass	0.200	Pass	0.102	Treat		0.000	WR vs TWR	
	TWR	Pass	0.422	2017	Fail	0.033			Year		0.001	WR vs CT	
	CT	Pass	0.399						Inter		0.056	TWR vs CT	
Ca	WR	Fail	0.001	2015	Pass	0.234	Fail	0.003	Treat	32.594	0.000	WR vs TWR	0.034
	TWR	Pass	0.095	2017	Pass	0.065			Year	70.033	0.000	WR vs CT	0.001
	CT	Pass	0.179						Inter	24.077	0.000	TWR vs CT	0.000
Cr	WR	Pass	0.091	2015	Fail	0.017	Fail	0.001	Treat	4.761	0.012	WR vs TWR	0.113
	TWR	Fail	0.005	2017	Pass	0.108			Year	58.516	0.00	WR vs CT	0.186
	CT	Fail	0.001						Inter	3.140	0.051	TWR vs CT	0.007
Cu	WR	Fail	0.001	2015	Pass	0.370	Fail	0.003	Treat	62.302	0.000	WR vs TWR	0.016
	TWR	Pass	0.763	2017	Pass	0.068			Year	34.259	0.000	WR vs CT	0.000
	CT	Pass	0.991						Inter	15.570	0.000	TWR vs CT	0.000
Fe	WR	Pass	0.372	2015	Pass	0.370	Pass	0.062	Treat	6.229	0.004	WR vs TWR	0.014
	TWR	Pass	0.650	2017	Pass	0.068			Year	12.409	0.001	WR vs CT	0.000
	CT	Pass	0.415						Inter	11.095	0.00	TWR vs CT	0.089
K	WR	Fail	0.001	2015	Pass	0.582	Fail	0.002	Treat	18.814	0.000	WR vs TWR	0.001
	TWR	Pass	0.186	2017	Pass	0.814			Year	15.132	0.000	WR vs CT	0.029
	CT	Pass	0.525						Inter	11.312	0.000	TWR vs CT	0.000
Mg	WR	Fail	0.025	2015	Pass	0.363	Fail	0.008	Treat	47.661	0.000	WR vs TWR	0.004
	TWR	Pass	0.845	2017	Pass	0.054			Year	26.355	0.000	WR vs CT	0.000
	CT	Pass	0.715						Inter	9.520	0.000	TWR vs CT	0.000
Mn	WR	Pass	0.896	2015	Fail	0.00	Pass	0.634	Treat	85.989	0.000	WR vs TWR	0.617
	TWR	Pass	0.921	2017	Pass	0.852			Year	6.291	0.015	WR vs CT	0.000
	CT	Pass	0.943						Inter	7.448	0.001	TWR vs CT	0.000
Na	WR	Pass	0.312	2015	Pass	0.868	Fail	0.013	Treat	3.981	0.024	WR vs TWR	0.465
	TWR	Pass	0.127	2017	Pass	0.108			Year	7.813	0.007	WR vs CT	0.006
	CT	Pass	0.250						Inter	0.893	0.415	TWR vs CT	0.044
Ni	WR	Fail	0.001	2015	Fail	0.000	Fail	0.009	Treat	7.878	0.001	WR vs TWR	0.596
	TWR	Pass	0.018	2017	Fail	0.013			Year	50.816	0.000	WR vs CT	0.000
	CT	Fail	0.004						Inter	2.738	0.073	TWR vs CT	0.002
P	WR	Fail	0.00	2015	Pass	0.119	Fail	0.008	Treat	35.142	0.000	WR vs TWR	0.011
	TWR	Pass	0.577	2017	Pass	0.596			Year	33.631	0.000	WR vs CT	0.000
	CT	Pass	0.326						Inter	8.992	0.000	TWR vs CT	0.002
S	WR		0.00	2015	Pass	0.899	Fail	0.002	Treat	33.842	0.000	WR vs TWR	0.006
	TWR	Pass	0.135	2017	Pass	0.120			Year	61.587	0.000	WR vs CT	0.000
	CT	Pass	0.165						Inter	16.072	0.000	TWR vs CT	0.000
Si	WR		0.014	2015		0.010	Pass	0.301	Treat	4.150	0.021	WR vs TWR	0.078
	TWR	Pass	0.090	2017		0.007			Year	19.957	0.000	WR vs CT	0.196

	CT	Pass	0.201						Inter	24.611	0.000	TWR vs CT	0.694
St	WR		0.010	2015	Pass	0.160	Fail	0.004	Treat	45.512	0.000	WR vs TWR	0.012
	TWR	Pass	0.566	2017		0.046			Year	47.275	0.000	WR vs CT	0.000
	CT	Pass	0.085						Inter	13.104	0.000	TWR vs CT	0.000
Ti	WR		0.005	2015		0.003	Fail	0.00	Treat	3.771	0.029	WR vs TWR	0.012
	TWR	Pass	0.060	2017	Pass	0.273			Year	10.734	0.002	WR vs CT	0.017
	CT		0.019						Inter	0.486	0.617	TWR vs CT	0.987
Zn	WR	Pass	0.249	2015	Pass	0.079	Pass	0.388	Treat	36.571	0.00	WR vs TWR	0.334
	TWR	Pass	0.428	2017	Pass	0.257			Year	8.847	0.004	WR vs CT	0..000
	CT	Pass	0.162						Inter	3.336	0.043	TWR vs CT	0.000
C	WR		0.001	2015	Pass	0.298	Fail	0.001	Treat	54.753	0.000	WR vs TWR	0.613
	TWR		0.004	2017	Pass	0.418			Year	293.65	0.000	WR vs CT	0.000
	CT		0.003						Inter	4.123	0.021	TWR vs CT	0.000
N	WR		0.00	2015	Pass	0.988	Fail	0.000	Treat	31.695	0.000	WR vs TWR	0.000
	TWR		0.00	2017	Pass	0.488			Year	710.78	0.000	WR vs CT	0.176
	CT	Pass	0.016						Inter	10.790	0.000	TWR vs CT	0.000

Table A.2. Porewater results of One-way ANOVA using factor of treatment site (cattail dominated: CT, wild rice:WR and Treated site: TWR). Significant difference was determined using Post Hoc analysis

Porewater Normality (All sites, All Depths, No water column)									
Parameter	Data Tests (P≥ 0.05)				ANOVA (P≤ 0.05)				LSD p-value
	Normality		Equal Variance						
	Results	P	Results	P	F	P	Sign.		
DOC	Pass	0.872	Pass	0.685	1.99	0.192	No	WR vs TWR	0.077
								WR vs CT	0.383
								CT vs TWR	0.310
Chloride*	Pass	0.785	Pass	0.175	9.544	0.06	Yes	WR vs TWR	0.010*
								WR vs CT	0.002*
								CT vs TWR	0.372
Nh3*	Fail	0.012	Fail	0.004	3.957	0.058	No	WR vs TWR	0.127
								WR vs CT	0.021*
								CT vs TWR	0.294
Nitrate*	Fail	0.00	Fail	0.008	1.075	0.381	No	WR vs TWR	0.570
								WR vs CT	0.179
								CT vs TWR	0.408
Calcium	Pass	0.179	Pass	0.139	5.112	0.033	Yes	WR vs TWR	0.069
								WR vs CT	0.012*
								CT vs TWR	0.307
Iron*	Pass	0.370	Pass	0.132	3.463	0.077	No	WR vs TWR	0.049*
								WR vs CT	0.991
								CT vs TWR	0.048*
Potassium	Pass	0.678	Pass	0.545	0.161	0.854	No	WR vs TWR	0.708
								WR vs CT	0.872
								CT vs TWR	0.594
Magnesium	Pass	0.283	Pass	0.095	5.813	0.024	Yes	WR vs TWR	0.173
								WR vs CT	0.008*
								CT vs TWR	0.087
Manganese*	Pass	0.357	Fail	0.000	1.181	0.350	No	WR vs TWR	0.503
								WR vs CT	0.159
								CT vs TWR	0.425
Sodium	Pass	0.092	Pass	0.363	1.501	0.274	No	WR vs TWR	0.121
								WR vs CT	0.539
								CT vs TWR	0.310
Sulphur	Pass	0.347	Pass	0.481	13.661	0.02	Yes	WR vs TWR	0.001*
								WR vs CT	0.129
								CT vs TWR	0.007*
Strontium*	Pass	0.170	Pass	0.235	4.304	0.049	Yes	WR vs TWR	0.046*
								WR vs CT	0.024*
								CT vs TWR	0.690

Zinc*	Pass	0.166	Pass	0.243	2.809	0.113	No	WR vs TWR	0.099
								WR vs CT	0.719
								CT vs TWR	0.054
Phosphates	Pass	0.046	Pass	0.246	2.117	0.176	No	WR vs TWR	0.141
								WR vs CT	0.771
								CT vs TWR	0.088
Total N*	Pass	0.046	Pass	0.309	2.180	0.169	No	WR vs TWR	0.111
								WR vs CT	0.098
								CT vs TWR	0.935
Total P	Pass	0.053	Pass	0.103	1.641	0.247	No	WR vs TWR	0.180
								WR vs CT	0.841
								CT vs TWR	0.131

Appendix B Pictures

Figure B.1 Mesocosm Schematic A) The peeper structure inserted into the sediment B) interval 10-0 within the water column and 0-10, 10-20,20-30 and 30-40 within the sediment C) individual dialysis water sampler and section

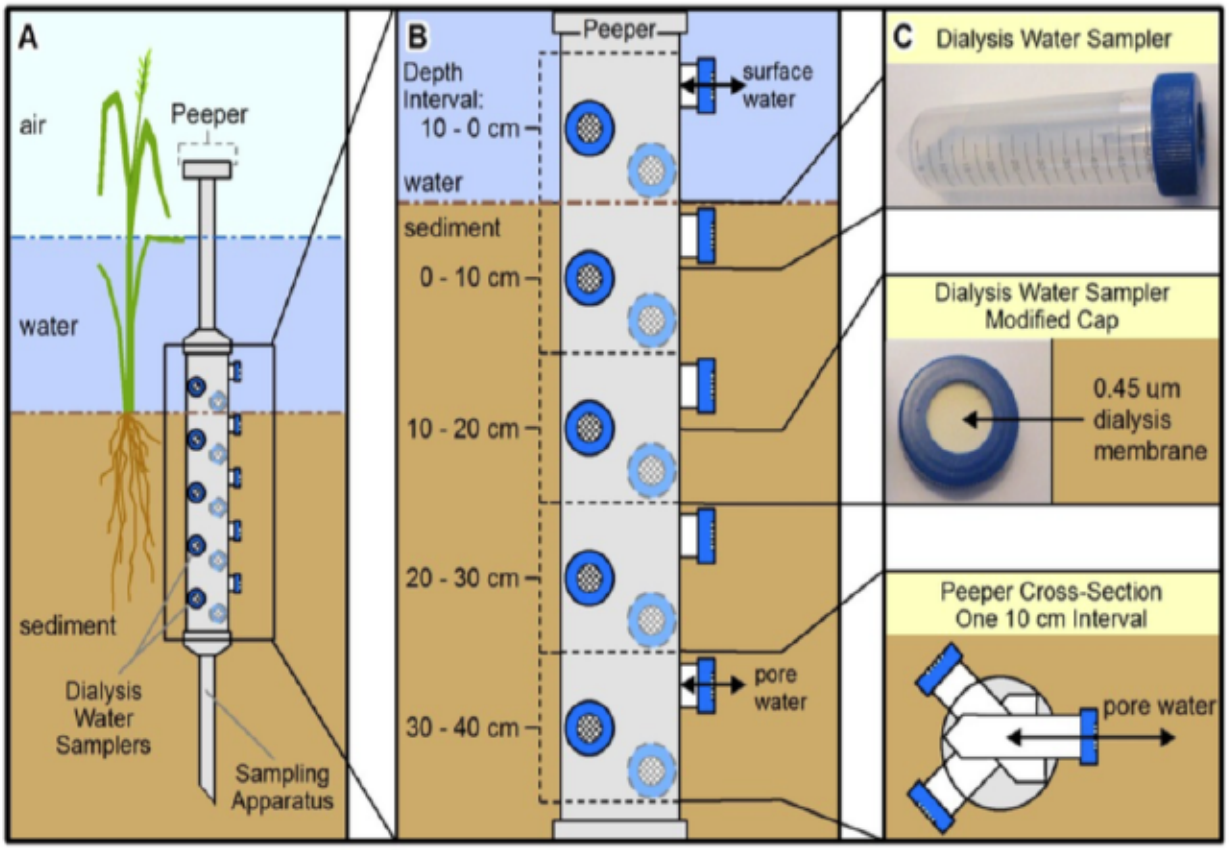

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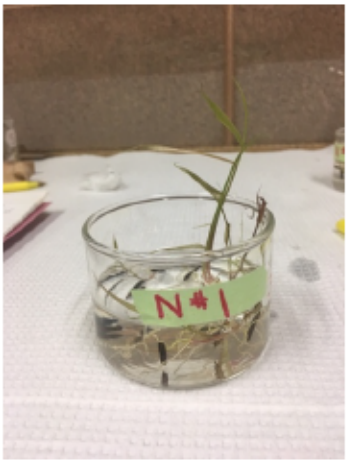
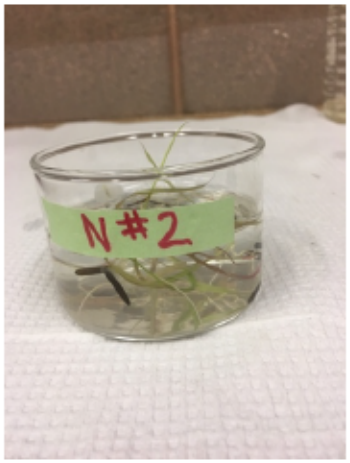
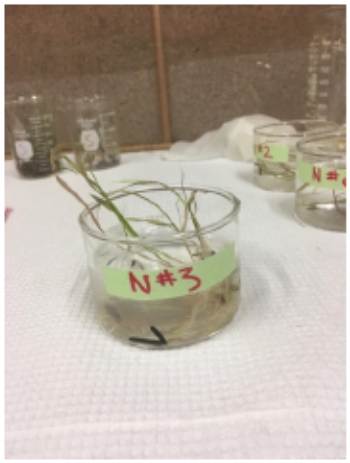
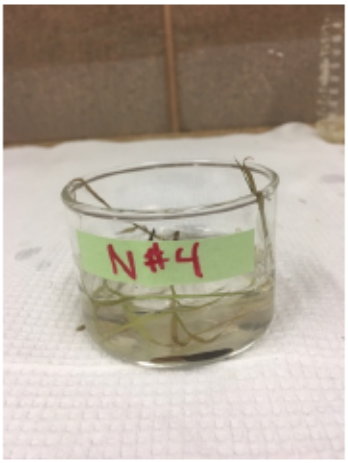
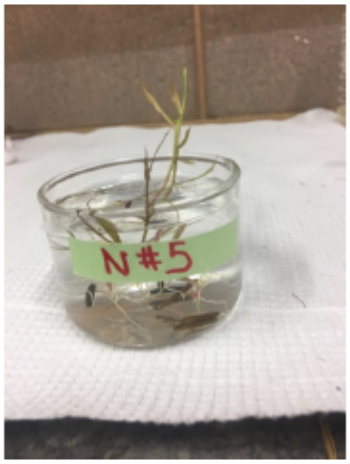
Figure B.2 Dissolved Oxygen Germination Trial Photos

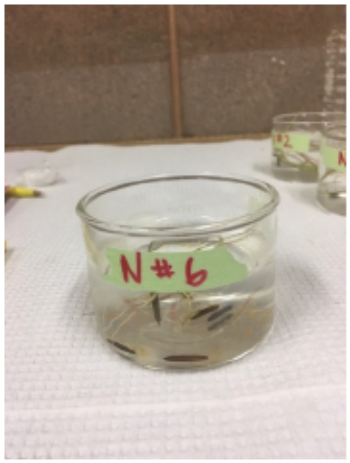


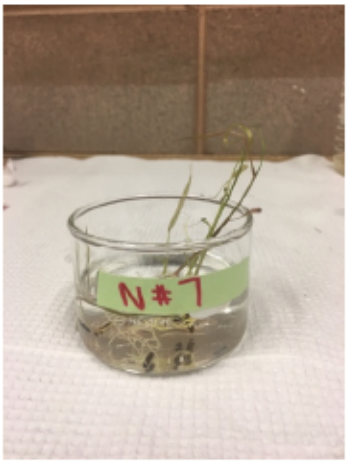


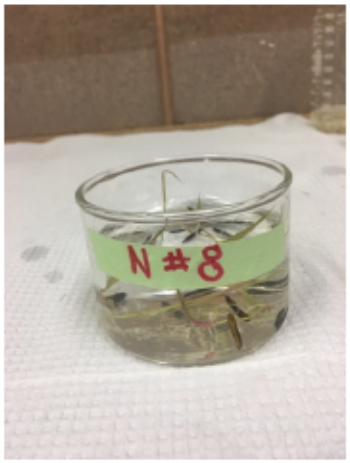


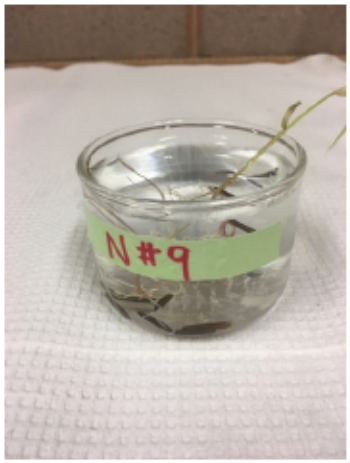


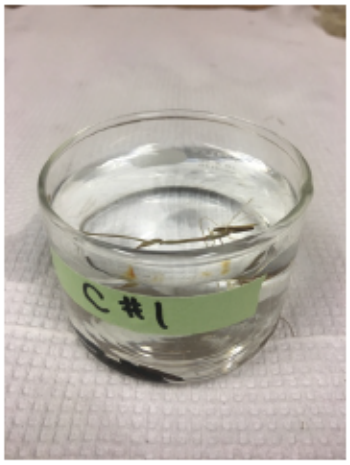








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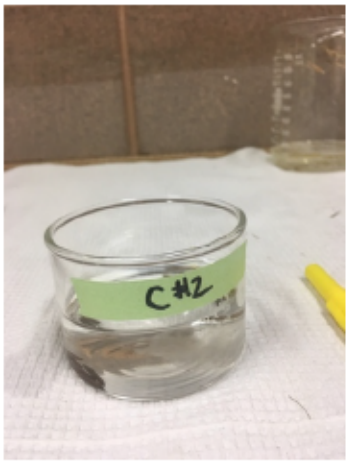
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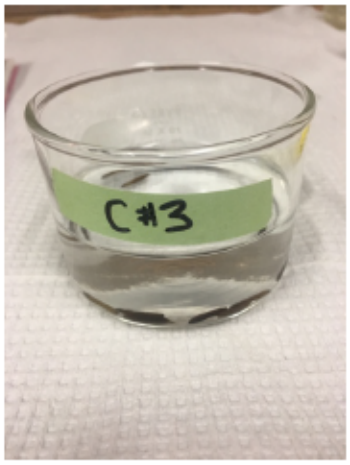
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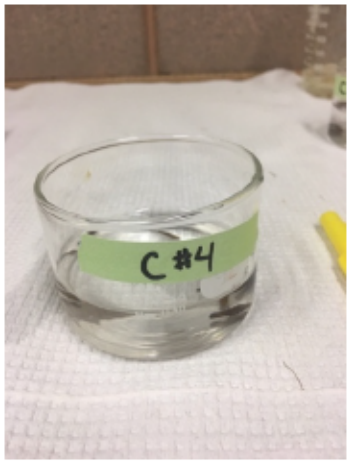
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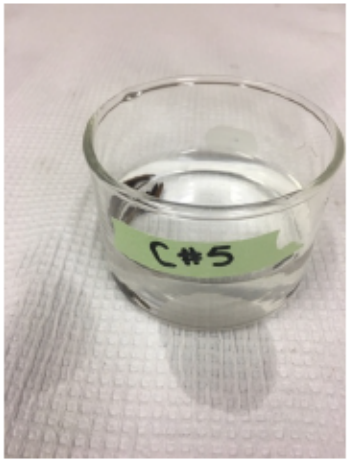
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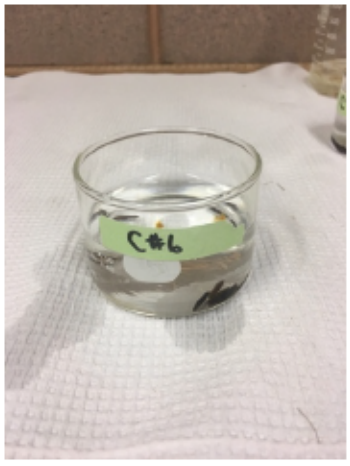
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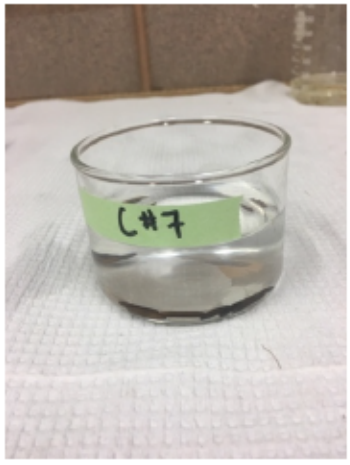
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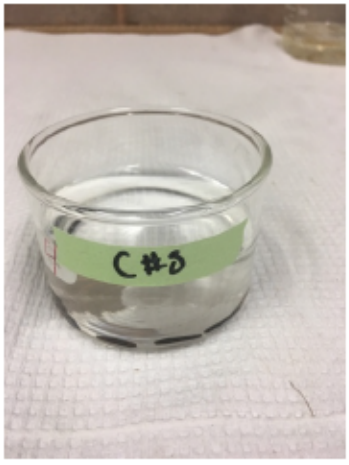
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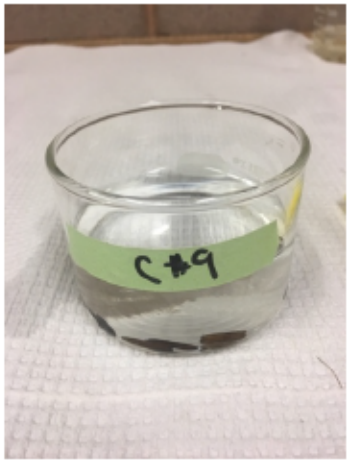
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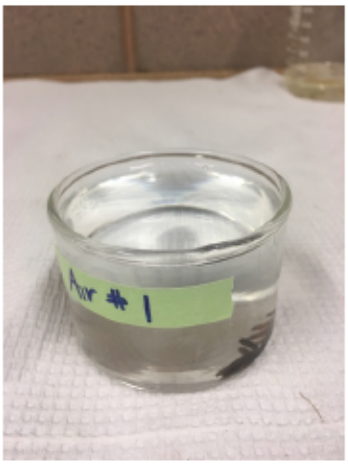
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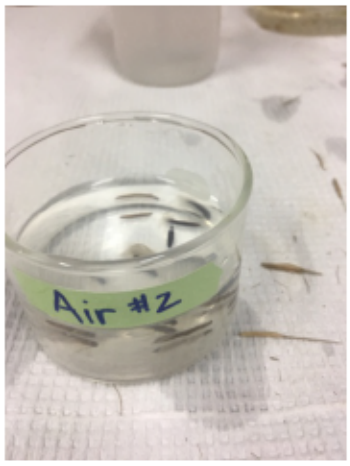
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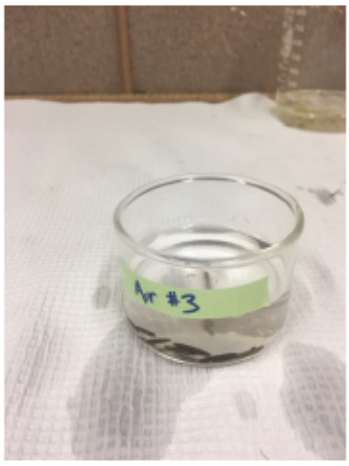
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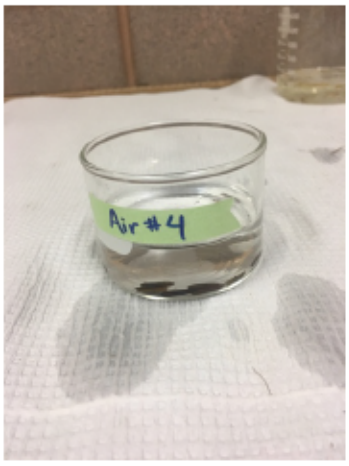
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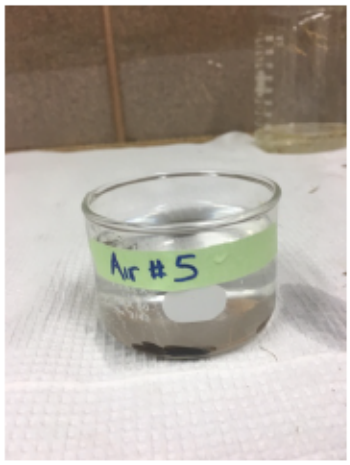
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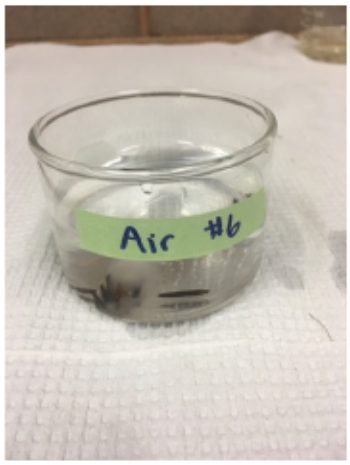
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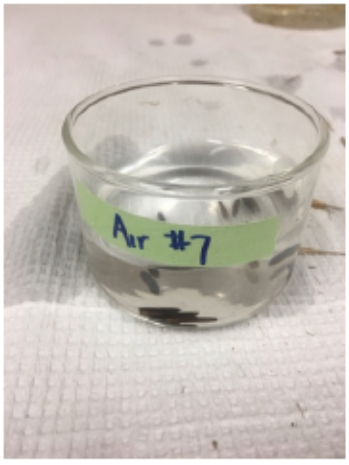
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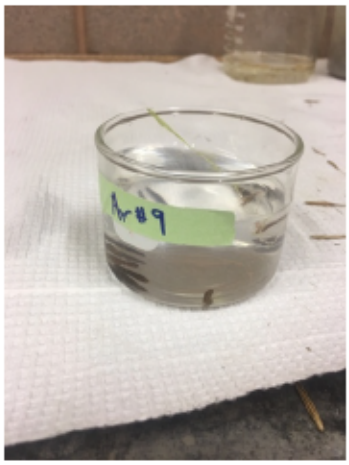
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