Are plant traits a practical indicator for monitoring ecological restoration projects?

by

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Author's Declaration

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

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Abstract

In order to restore ecosystems, we must have reliable monitoring indicators to identify the success of ecological restoration, and make effective management decisions. Indicators must be simple and inexpensive to measure to allow for practical industry use, account for ecological and environmental change, and provide direction to ecosystem managers. Trait-based monitoring approaches have been proposed as an alternative measure to traditional vegetation monitoring indicators (e.g. measures of species diversity), as they provide more information about the function of an ecosystem and their abiotic-biotic interactions. The literature indicates that plant functional traits, which explain how an organism acquires, processes and invests in resources, may be an ideal monitoring indicator.

I examined the practical application of plant traits to assess restoration success in a phragmites managed Great Lakes Coastal Wetland, in the face of significant management and ecological variability. Two traits often cited in the literature, specific leaf area and leaf dry matter content, were measured in open marsh and three created ponds of different ages, and in plots treated with glyphosate and plots left un-treated. Non-parametric Mann-Whitney U and Kruskal Wallis tests were used to compare the community weighted mean trait values of ponds and open marsh, and between herbicide treatment groups. Notable results included an increase in specific leaf area after herbicide treatment, and a decrease in leaf dry matter content, suggesting that traits promoting biomass production may be favoured after herbicide treatment. This is consistent with previous studies comparing weighted means after management action, as well as in successional trait studies. However, it will be important to consistently monitor invasive species presence and management actions, in order to confirm that trait changes are due to herbicide treatment. No statistically significant differences were found between ponds and open marsh for specific leaf area, and leaf dry matter content only differing significantly in one pond. Possible explanations for this lack of change and the variable changes between ponds include intraspecific variation, environmental factors like water level fluctuations, and management variation between ponds.

To conclude, the use of leaf traits, and plant traits in general, are possible as a restoration monitoring measure, but their use is highly site specific. They are best suited in situations where variability is low; where they can be compared against a measureable environmental gradient or a well identified management regime, two factors that do not always exist in a practical resource management context. Time also plays a role in the application of traits to restoration projects; the more traits that can be measured, and more resources allocated to the understanding and application of additional functional diversity indices, the more valuable their use. Future research and management opportunities include the integration of trait-based monitoring measures into diversity monitoring regimes, the exploration of traits as a restoration design measure to combat exotic species invasions, and testing ecological indicator criteria on other key traits (e.g. belowground traits, regenerative traits) to asses their potential as monitoring measures for future restoration project monitoring.

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1. The Practice & Measurement of Outcomes in Restoration Ecology

1.1 Why Restoration Ecology?

The Millennium Ecosystem Assessment (2005) identified that one third of global ecosystems have been degraded by a variety of human-induced actions, including fragmentation, unsustainable harvesting, pollution, and exotic species introduction (MEA, 2005; Suding, 2011). Considering these impacts, simply conserving the landscape is not enough to reverse much of this environmental damage, and the restoration of degraded ecosystems has become a key factor in conservation practices and global conservation strategies (Brudvig, 2017; McDonald et al., 2016). For example, the Convention on Biological Diversity seeks to restore 15% of degraded ecosystems by 2020 to fight climatic change and desertification, and the United Nation's Sustainable Development Goals, within their 2030 Agenda for Sustainable Development, includes terrestrial ecosystem restoration as a key factor in ecosystem management and the prevention of degradation (Gann et al., 2018; United Nations, 2018). Though restoration is a global priority, the 'why' and 'how' to best restore ecosystems is an elusive question; approaches to and motivations that drive ecological restoration are debated intensively in the peer reviewed literature (Hertog & Turnhout, 2018; Higgs et al., 2018). To use restoration in many of these global initiatives to combat degradation, we need to understand what motivates restoration, and how to effectively restore ecosystems (Brudvig, 2017; Hagger et al., 2017). This includes a firm understanding of the science behind restoration, as well as clear mechanisms by which restoration can inform resource managers (Suding, 2011).

Defined by the Society for Ecological Restoration's International Standards for the Practice of Ecological Restoration (SER Standards) as '*the process of assisting the recovery of an ecosystem that has been degraded, damaged or destroyed,'* ecological restoration can drastically improve degraded systems (McDonald et al., 2016). Restoration has become a common practice amongst government, private and non-profit sectors (Clewell & Aronson, 2006; Henry et al., 2018). Over time, it has bridged the gap between conservation and industry, to include groups that may have been previously excluded (e.g., industrial resource extraction, corporate groups) (Higgs, 1997). Restoration is primarily used to recover biodiversity and re-establish ecosystem services (Brudvig, 2017; Holl & Aide, 2011; Meli et al., 2014; Suganuma & Durigan, 2015; Wortley et al., 2013). The restoration of biodiversity could include guiding ecological recovery, compensating for habitat loss and re-establishing ecosystem resilience (Suding, 2011). Restored ecosystems can provide many services that benefit society, including clean water, healthy soils, and clean air (e.g. erosion control, floodwater storage, water quality improvements, pollution management, carbon storage) (Ehrenfeld, 2000; McDonald et al., 2016; Rey Benayas et al., 2009). Furthermore, healthy, functioning ecosystems can aid in the mitigation of climate change and natural disasters (McDonald et al., 2016).

Biodiversity recovery and ecosystem service provisioning are large goals, and there have been several different reasoning frameworks for why we restore ecosystems within these broad fields (e.g. Clewell & Aronson, 2006; Suding, 2011; Suding et al., 2015). In general, restoration should increase ecological integrity, be sustainable long term, be completed with consideration for both the past and future, and benefit and engage society (Suding et al., 2015). While the ecological principles by which we restore are crucial in meeting these targets, proper goals and objectives in a restoration project are equally important in the restoration process (Higgs, 1997). Similarly, we make decisions based on both ecological and technical principles, as well as our own individual cultural experiences (Higgs, 1997). Regardless of how the motivation for ecological restoration is classified, one key theme emerges: the inclusion of multiple viewpoints in determining why one wishes to restore. The variety of motivational drivers exists due to different priorities amongst the different stakeholders in restoration project, who all have different values (Hagger et al., 2017; Higgs, 1997; Suding, 2011). These values could be scientific, historical, political, cultural, or social in nature, and it is important to consider this diverse value system as we make decisions about how we should be restoring a given ecosystem (Hagger et al., 2017).

1.2 Measuring Restoration Outcomes: The Selection of Monitoring Indicators

Even in 2019, there is still a gap and a need to identify key indicators that can determine how we monitor, assess restoration goals and manage ecosystems to recover biodiversity and ecosystem services (Dale & Beyeler, 2001; McAlpine et al., 2016; Suganuma & Durigan, 2015). Success indicators can be used in several ways; they can act as a warning sign for ecosystem change or for future degradation of ecosystem health, and they can provide insight in the success of a restoration project (Dale & Beyeler, 2001; Seilheimer et al., 2009). The most common indicators used for measuring restoration success are ecological in nature. Wortley et al. (2013) conducted a review of restoration literature and cited that 94% of surveyed articles included ecological attributes. Indicators should consider the structure, function and composition of the ecosystem (Chang et al., 2016; Dale and Beyeler, 2001; Ruiz-Jaen & Aide 2005a, Ruiz-Jaen and Aide 2005b). Ecological indicators are therefore broken into vegetation structure, species diversity and abundance, and ecological processes (Wortley et al., 2013). They can focus on key species that act as indicator, keystone, or at-risk species (Suganuma & Durigan, 2015). Vegetation recovery is also a common tool to evaluate restoration success, but it is important to consider vegetation structure, species diversity, and ecosystem processes in the recovery process (Ruiz-Jaen & Aide, 2005a; Ruiz-Jaen & Aide, 2005b). It is important to note that restoration indicators can also be selected to reflect socioeconomic and ecosystem service success measures, though this is less commonly selected as an success measure (Rey Benayas et al., 2009; Wortley et al., 2013).

Indicators work best when situated within a restoration project that has clearly defined goals, objectives and timelines, that correspond to an ecosystem's characteristics (Brudvig, 2017; Gann et al., 2018). The ideal is to allow lead time for indicator selection to alert a manager to the changes occurring in the ecosystem to be restored (McDonald et al., 2016) and (predictably) respond to stress and account for ecosystem complexity (Dale & Beyeler, 2001). Indicators also need to provide direction for ecosystem managers, and be simple and inexpensive to measure (Dale & Beyeler, 2001; McDonald et al., 2016; Suganuma & Durigan, 2015). Comprehensive indicators will be usable across temporal and spatial scales; this can be as simple as being practical and usable for long periods of time (Dale & Beyeler, 2001; McAlpine et al., 2016).

1.3 Challenges in Restoration Project Design and Monitoring Indicator Selection

1.3.1 Theoretical Challenges

Choosing the right indicator to assess ecological restoration is challenging for several reasons. A significant debate in restoration monitoring literature concerns the inclusion of a reference site in restoration assessment. Historically, it has been common in both ecological monitoring and restoration practice that one should compare a restored ecosystem to a reference ecosystem that would represent ideal, or near original conditions for a given ecosystem. This reference system should be in the same geographical region, near the restoration site, and experience similar disturbance conditions (Ruiz-Jaén & Aide, 2005b). Numerous studies state this as a key requirement, or at least strongly encourage a reference ecosystem to be used in restoration work (Ehrenfeld, 2000; Kentula, 2000; Ruiz-Jaen & Aide, 2005a; Ruiz-Jaén & Aide, 2005b). In a restoration indicator literature review by Wortley et al. (2013), 74% of articles captured used a reference or control in their study. The SER Standards (McDonald et al., 2016) also supports the use of a reference system as one of six key concepts for best practice.

The concept of a static reference system has been criticized in recent years due climate change, invasive species and ecosystem service research (Higgs et al., 2018). Environmental changes create conditions in which a reference system may not be found near a restoration site. Environmental changes also increase the number of alternative stable states that an ecosystem could return to, which could be significantly further from its ‘original state,’ making the identification of a reference site more difficult (Murphy, 2018). Restoration now includes innovative research fields of green infrastructure and agro-ecosystems, which also may not fit within the requirement of a reference site (Higgs et al., 2018). Higgs et al. (2018) stresses that a more open and flexible process within restoration goals and objectives is required to ensure the success of restoration in a changing world. Suding et al. (2015) also calls for flexibility when referring to the use of history to guide restoration, as we face future environmental changes. Gann et al., (2018) argues that the SER Standards encourage recovery towards a reference ecosystem ‘insofar as possible’ and that the Standards encourage the consideration of environmental changes when conducting restoration. As such, the use of a reference site remains an actively discussed concept within the practical study of restoration ecology.

Novel ecosystems, which are defined as a naturally occurring ecosystem that has been pushed past the threshold which they could return to their original or natural state, are an equally debated concept within the restoration community (Hobbs et al., 2014). Largely caused by invasive species and climate change in many cases, novel ecosystems contain ecological characteristics that haven’t previously existed, making the reference ecosystem target impossible to meet without significant resource and cost requirements, and the need for a more broader definition of ‘restoration’ (Hertog & Turnhout, 2018; Hobbs et al., 2009; Miller & Bestelmeyer, 2016).

Hybrid systems could already be the dominant form of ecosystem, and likely more dominant in the future, where less damaging but hard to eradicate non-native species could be included in an acceptable ecosystem state and resulting restoration project (Hobbs et al., 2009). As we experience extreme global environmental changes, we may need non-native species for ecological systems to continue to provide ecosystem services (e.g. pollinator habitat, water filtration, recreation) (Hobbs et al., 2009; Standish et al., 2014). Guiding documents such as the SER Standards do acknowledge that irreversible damage can occur in an ecosystem, largely attributed to environmental changes (Miller & Bestelmeyer, 2016). Those who argue for the novel ecosystem concept stress that novel ecosystems have the potential to allow better goal development and subsequent management than considering historical reference sites as an end goal (Hobbs et al., 2014). Those against the novel ecosystem concept argue that a true ‘irreversible’ threshold may not exist between hybrid and novel states (Miller & Bestelmeyer, 2016). They also critique the term ‘hybrid ecosystem’ as vague and difficult to define, which causes it to be ignored as an ecosystem state in literature (Miller & Bestelmeyer, 2016). Those opposed also argue that they threaten the current policy and management implications surrounding restoration projects and places less focus on keeping natural conservation assets

(Hobbs et al., 2014). Others worry that allowing novel ecosystems into the discussion can create loopholes to allow corporate and government agencies to cause further environmental degradation (Hobbs et al., 2014; Murphy, 2018). Regardless of the side one sits on the debate of novel ecosystems and how we classify and manage these systems, they will be a strong consideration for restoration design and indicator selection.

1.3.2 Practical Challenges

Restoration projects overall, and specifically post restoration monitoring, are costly to implement (Cui et al., 2009; Wortley et al., 2013). In the context of novel ecosystems, cost plays a role in their development and management; in theory, much environmental degradation is reversible, but managers are often limited by financial, technical, social and institutional barriers (Hobbs et al., 2014). This is especially common for private, NGO and government groups, who often do not have the ability to measure more than one indicator when assessing restoration (Henry et al., 2018). From a project implementation perspective, policy development for the financial and regulatory components of ecosystem restoration is largely non-existent, because of the unknowns surrounding restoration monitoring and assessment (Chaves et al., 2015). If money is to be allocated to restoration initiatives, we need clear mechanisms by which we can evaluate success, including cost-effectiveness, socioeconomic benefits and ecological outcomes (Chaves et al., 2015).

Time also plays a role in indicator selection. Ecosystem recovery time varies by ecosystem and this process can take significant amounts of time (Wortley et al., 2013). Holl and Aide (2011) estimate that aquatic environments can take up to ten years to recover, and some forest ecosystems can take up to forty, depending on ecosystem and degradation characteristics. It is a common societal expectation for an environmental problem to be fixed immediately with limitless funding, which are two unrealistic goals (Holl & Aide, 2011). Monitoring itself can be a time-consuming step to implement, and its success can depend on the experience of the individuals conducting the monitoring (Cui et al., 2009). Finally, ecological restoration is a relatively new field of study (Holl & Aide, 2011), and many components of ecosystem structure and complexity are not always captured in simplified monitoring protocols. Monitoring programs and restoration are often conducted without clear project goals or monitoring protocols, which should be clearly outlined prior to the start of a study or project in order to maximize the use and relevance of collected data (Dale & Beyeler, 2001; Kentula, 2000; Wortley et al., 2013). When indicators are not properly defined, and they are not established under clear objectives, this can negatively impact the strength of a resulting management protocol (Dale & Beyeler, 2001).

1.4 Plant Functional Traits May Be a Useful Indicator in Restoration Ecology

1.4.1 What are Functional Traits?

Plant functional traits are part of a larger discussion of functional diversity, which is defined by the '*value and range of functional traits of the organisms present in an ecosystem'* (Standish et al., 2014). A rather ancient concept (e.g. spanning the era from Theophrastus to Darwin), functional diversity became commonly accepted in the 1950s/1960s to explain how plants utilized resources and how they could be grouped according to resource use (Laureto et al., 2015). By the 1990s, human-driven environmental change had become more prevalent, and research in plant functional diversity moved towards how functional diversity could be used in an efficient and ecologically relevant way to explain relationships between plant communities and ecological function (Laureto et al., 2015).

Functional ecology has had many names in the past century, from 'comparative ecology' to the 'functional ecology' to 'trait based ecology' and 'plant functional traits' (Shipley et al., 2016). I will use the latter two terms in this thesis where functional traits are defined as the characteristics of an organism (e.g. morphological, physiological or phenological) that explain how plants acquire, process and invest in resources, and impact the overall fitness of a plant (Díaz et al., 2004; Pérez-Harguindeguy et al., 2013). Functional traits not only represent how species acquire resources, but also how they reproduce, disperse and respond to environmental changes (Funk et al., 2008). There are several key characteristics of trait-based ecology:

- Trait based ecology considers the phenotypic characteristics of a plant over their phylogenic characteristics (Shipley et al., 2016)

- Trait-based ecology compares trait values of multiple species and ecosystems in order to determine general environment-plant related trends to make generalized conclusions about the state of an ecosystem (Shipley et al., 2016)
- Trait-based ecology compares trait values against environmental gradients (biotic, such as competition and predation, or abiotic such as climate and resource availability) in order to determine how environmental conditions influence community assembly (response traits) and how existing traits influence environmental conditions (effect traits) (Funk et al., 2008; Lavorel & Garnier, 2002; Shipley et al., 2016)
- Trait-based ecology assumes that trait values at smaller spatial scales directly influence trait values at larger spatial scales (Shipley et al., 2016)

Functional traits can be addressed in both flora and fauna, but plant functional traits are a common research focus as they make up 99% of living material in terrestrial systems and are key components in global atmosphere and climate dynamics (Garnier et al., 2016; Shipley et al., 2016). While classifications of plant functional types have not been fully standardized within the academic community (Lavorel et al., 2007), several large lists of plant traits exist in literature, built upon several ecological theories, attempting to identify universal traits that help researchers choose which traits to measure (e.g. Cornelissen et al., 2003; Lavorel et al., 2007; Pérez-Harguindeguy et al., 2013; Weiher et al., 1999). The peer literature has devised protocols to measure ‘soft’ traits, which are easy and quick to measure; in contrast, ‘hard traits’ are ones that may not be measured consistently across regions or may take more time to measure (Lavorel et al., 2007; Lavorel & Garnier, 2002). Soft traits can also be used as a proxy for hard traits, which provide information about both response (species response to both abiotic and biotic characteristics in an ecosystem) and effect (a species trait influence on ecosystem processes) components of plant traits, but are harder to measure (Funk et al., 2008; Lavorel & Garnier, 2002; Weiher et al., 1999).

‘Response traits’ are those that easily respond to environmental changes (Laughlin, 2014). Two theories support response traits; species that have similar functional traits will not occupy the same niche space in an ecosystem (limiting similarity) and species can be competitively excluded because some species exhibit traits that are better than others (e.g. nutrient or light acquisition) (Laughlin, 2014). ‘Effect traits’ are possessed by organisms that influence the function of an ecosystem, and are supported by the mass ratio hypothesis, which suggests that the most dominant species contribute the most to a community (Grime, 1998; Laughlin, 2014). Soft traits can be broken up into four general groups; whole-plant traits, leaf traits, stem and below ground traits, and regenerative traits (Drenovsky & James, 2010; Wright et al., 2004). Researchers have sought to develop core lists of plant traits that are universally useful for assessment against environmental change (e.g. Weiher et al. 1999). Others propose trait selections based on plant life cycles (e.g. how plants establish a healthy population), known as plant ecological strategies (Pérez-Harguindeguy et al., 2013).

Plants can be categorized along a spectrum of growth defined by resource capture and resource conservation; how plants obtain carbon during photosynthesis, and how they store it and create plant tissue. For example, on one side of the spectrum, plants produce thin, less dense leaves, which allow for significant growth and resource acquisition but risks resource loss through herbivores and environmental disturbances. The opposite side of the spectrum has plants that exhibit dense leaves that are well protected against disturbance, but plant growth and resource capture is less (Drenovsky & James, 2010; Wright et al., 2004). In spite of the theory driving trait selection, the selection of which traits to measure is highly dependent on one’s ecological question, project scale and resources available (Perez-Harguindeguy et al., 2013).

1.4.2 Why are Functional Traits A Good Monitoring Indicator?

Plant traits that can identify changes in ecosystem function have become increasingly important in the past ten years, as focus has been placed on ecosystem services, which depend on ecosystem function (Standish et al., 2014). Trait based ecology has also become increasingly used to predict and explain vegetation community responses to environmental changes (biogeochemical cycles, invasion resistance, disturbance regimes) (Standish et al., 2014). For this reason, the use of plant functional traits has become a widely-discussed indicator of ecosystem dynamics as opposed to more traditional diversity measurements for plant communities (Brudvig, 2017). Trait-based approaches in ecology have been used to discuss the relationships between species assembly along abiotic gradients, community assembly characteristics and scaling individual organism function up to the ecosystem functional level (Garnier et al., 2016, p.5). It has been promoted as a promising measure for its ability to generalize and predict community structure and function and has been increasingly used in ecological literature (Stefanik & Mitsch, 2012).

When conducting plant community monitoring post-restoration, monitoring is often focused on taxonomic measurements to measure diversity, and whether biodiversity has changed after a site has undergone restoration (Mouchet et al., 2010). Examples of diversity measurements include percentage cover of groundcovers and native species, as well as species richness and related diversity (Mouchet et al., 2010). One of the assumptions made in biodiversity measurements and indices is that all species are equal, and only differ in their relative abundance in a community (Swenson, 2011). However, different species contribute in different ways to ecosystem processes, and as a result, ecosystem services (Suganuma & Durigan, 2015). Simply quantifying the names and numbers of species does not provide any information about their function (Kyle & Leishman, 2009). Swenson (2011) argues that biodiversity in an ecosystem is not just species diversity, but also functional diversity. Two ecosystems could have similar species diversity, but completely differ functionally, resulting in significantly different biodiversity overall. Species diversity has also been critiqued as an indicator of restoration success, as it is hard to predict over time (Engst et al., 2016; Suganuma & Durigan, 2015). The use of diversity when assessing ecosystem resilience has also been critiqued for highly disturbed systems (Suganuma & Durigan, 2015). The species composition of a restored area can also differ from what was present in the past, or what is present in a selected reference site (Kyle & Leishman, 2009). Species richness measurements can also be a time consuming indicator to collect and accuracy can be questionable, depending on the identification skills of the data collector (Standish et al., 2014).

While functional traits may not provide direct information regarding an ecological or environmental phenomenon, they can be used as a proxy to interpret ecological and environmental impacts on an ecosystem (Engst et al., 2016). Trait based approaches can also provide more information about a system than just looking at one target species, an alternative monitoring approach (Engst et al., 2016). Functional traits have the potential to provide a more in-depth explanation of the restored state of an ecosystem, as they can examine the biotic-abiotic interactions of species with their environment (Cui et al., 2009; Wieten et al., 2012).

Pragmatically, traits have been stated to be easy to observe and measure, able to be used across a range of spatial scales and respond to environmental changes (Garnier et al., 2007; Lavorel et al., 2007). The plant trait manuals in literature (Cui et al., 2009; Wieten et al., 2012) also acknowledge that their proposed measurement protocols are easy and inexpensive to measure, in addition to being ecologically informative. As such, plant traits have the potential to fulfill much of the criteria set out by Dale and Beleyer (2001) of an ideal ecological indicator.

1.5 Wetland Ecosystems

Alarmingly, over 50% of global wetlands have been destroyed, and in certain Southern Ontario regions, up to 95% of Great Lakes coastal wetlands have been lost (Cui et al., 2009; Wieten et al., 2012). Urbanization and agriculture are the two main threats to wetlands and the ecosystem services that they provide by changing shoreline structure and water levels, causing nutrient and sediment loading, dredging and encouraging the establishment of invasive species (Cvetkovic & Chow-Fraser, 2011; Meli et al., 2014; OMNRF, 2017b).

Because of this significant loss, the assessment of remaining wetlands has become a top conservation priority, with wetland restoration as a crucial part of conservation practices (Meli et al., 2014; Uzarski et al., 2017). Ontario has recently made commitments towards wetland conservation and restoration, in order to combat threats against wetland health. The 2017 update of the Wetland Conservation Strategy for Ontario aims for a no-net loss target for Ontario's wetlands, and to create a policy to enforce this goal (OMNRF, 2017a). Specifically, they seek to achieve a no net loss of wetlands in areas where loss has been the greatest by 2025, and by 2030 to achieve a net gain in these areas. One of the considerations outlined in the development of the No-net loss policy is to 'establish monitoring requirements to ensure that wetland functions are restored' (OMNRF, 2017a). Restoration is also cited in additional Great Lakes remediation efforts as an important step in overall lake health, such as the Lake Erie Action Plan (MOECC, 2018).

Wetland restoration broadly involves the manipulation of hydrology, biology or soil, and is commonly completed in order to re-establish native vegetation for wildlife, often waterfowl species (Cui et al., 2009; Mitsch & Wang, 2000). This can include a multitude of methods, including the manipulation of hydrological regimes and water quality, altering wildlife habitat, vegetation composition and structure (e.g. invasive species exclusion), plant diversity, biomass, and soil microbial communities’ (Cui et al., 2009; Mitsch & Wang, 2000; Zhao et al., 2016). When a wetland is being assessed post restoration, monitoring consists of hydrology, vegetation and soil (Stefanik & Mitsch, 2012). Regardless of past efforts, there is a general need to increase the amount of post restoration monitoring in restored wetlands. Restoration monitoring is often completed within 5 years, but wetlands may require up to 15-20 years to return to a vegetation community like an unrestored wetland (Stefanik & Mitsch, 2012).

Wetland monitoring can be conducted in a variety of ways and can incorporate a variety of biotic and abiotic characteristics. They often include indices that focus on water quality, vegetation and vertebrates (Uzarski et al., 2017). Indexes of biotic integrity (IBI) are common, which incorporate multiple community characteristics to identify ecosystem change, often due to anthropogenic disturbances, and are used both in research and in industry monitoring (Rooney et al., 2012, Miller et al., 2006, O'Reilly et al., 2011). For plants, IBIs can include percent cover of annual, nonnative or invasive species, or Floristic Quality Assessments (FQA), which use the number and type of plant species in a given community to determine wetland health (Miller et al. 2006, Albert, 2008). Where region wide IBIs are more challenging to quantify, Great Lakes basin-wide programs focus on fewer measurements; The Great Lakes Wetland Monitoring Protocol uses the percent cover of invasive species, submerging and floating plant cover, and the calculation of the Floristic Quality Index (FQI) and coefficients of conservatism (C) (Albert, 2008; Lawson, 2004). The FQI uses coefficients of conservation values, which assign numbers (0-10) to specific species, depending on their resilience to disturbance and degradation, as well as their association to natural habitats (Freyman et al., 2016). C values are typically assigned to every species in a given geographical area; C values are then averaged and the FQI is calculated by weighting the average C value by species richness (Freyman et al., 2016). In general, low C values indicate species that tolerate anthropogenic disturbances, while higher C values identify species that are more sensitive, and are found in habits that have not experienced a high frequency of disturbance or anthropogenic change (Freyman et al., 2016). While these large-scale protocols are necessary to assess large-scale conditions, they are not always realistic regarding the time and resource requirement for such a large undertaking, and whether these protocols can be carried out by smaller non-government groups at smaller scales.

1.6 Using Traits in Restoration Ecology Projects - Action on the Ground

Measures of ecological function are becoming an increasingly used indicator in restoration monitoring, largely attributed to an increased number of mature restoration sites (Wortley et al., 2013). While the concept of plant traits is not new, the application of trait-based ecology to restoration work, especially regarding practical application, is a relatively new concept and is being proposed as a component of restoration projects in several different ways. Some have proposed predictive trait-based approaches in restoration project design, in order to set proper objectives to restore ecosystem resilience, as an alternative to the use of a reference system (Cadotte et al., 2015; Funk et al., 2008; Laughlin, 2014; Laughlin et al., 2017). For example, trait-based approaches have been used in restoration work to combat invasive species by selecting native species that have similar traits to the invading species (Funk et al., 2008). Others have sought to use traits to measure restoration success, through use as a monitoring indicator (D'Astous et al., 2013; Engst et al., 2016; Gondard et al., 2003; Kyle & Leishman, 2009; Sandel et al., 2011; Zirbel et al., 2017).

However, challenges exist with applying experimental research to industry-led restoration. Many studies are conducted using a significant number of field sites. For example, Zirbel et al. (2017) utilized 29 restored prairies in their study, with significant funding sources over long periods of time, characteristics that are rare in the resource management world, especially for monitoring protocols.

This thesis sought to assess not only the scientific, but also the practical application of using traits to conduct local scale restoration monitoring, an evaluation that is not commonly assessed in trait-based literature. If traits can perform ecologically as a monitoring indicator and fulfill the practical requirements of being simple, inexpensive and easy to measure, they may hold a place in the future of small-scale restoration monitoring and subsequent resource management decisions.

In the Great Lakes region of Southern Ontario, there are over 2,000 wetlands, many of which are marshes and play a significant role in the lifecycle of Great Lakes fishes (Cvetkovic & Chow-Fraser, 2011). They provided habitat for invertebrates, birds, turtles amphibians and, in northern regions, moose and bear (Cvetkovic & Chow-Fraser, 2011). In addition to supporting biodiversity, wetlands also provide ecosystem services. Categorized by the Millennium Ecosystem Assessment, these ecosystem services fit into supporting (e.g. habitat), provisioning (e.g. climate regulation), regulating (e.g. water quality) and cultural (e.g. recreation) benefit categories (MEA, 2005; Meli et al., 2014). Wetland ecosystems are an integral component in the landscape and contribute to both biodiversity and ecosystem services (Meli et al., 2014). Wetland ecosystems are an actively managed and restored system in Southern Ontario, with a great deal of ecological and ecosystem service value, which makes them an ideal system for such a case study.

The research goals of my thesis are to:

1. Complete the first test of whether plant functional traits have ecological potential as a site-specific restoration success measure in a Great Lakes Coastal Wetland, despite variation within an actively managed, novel ecosystem
2. Determine whether plant traits can be monitored from a practical perspective – does it satisfy criteria for an effective indicator?
3. Provide direction for future restoration initiatives in the Crown Marsh, a Great Lakes Coastal Wetland in Long Point, Ontario.

2.Testing the Utility of Plant Traits in Ecological Restoration Projects (Long Point Crown Marsh, Ontario, Canada)

2.1 Site Selection

The initial sampling universe was large for a trait-based wetland monitoring study. Potential field sites were arranged with the Ontario Ministry of Natural Resources (OMNRF), Royal Botanical Gardens (RBG) and Credit Valley Conservation Authority (CVC), in the fall of 2016 and winter of 2017. These sites were in Long Point (Port Rowan), Hamilton, and Mississauga, all within Southern Ontario (Figure 1). In April and May, 2017, all sites were visited to assess accessibility; both RGB and CVC sites were too deep for to access. RBG wetlands were also filled in with vegetation and lacked open water, which was dissimilar to both CVC and Long Point sites, so these sites were eliminated.

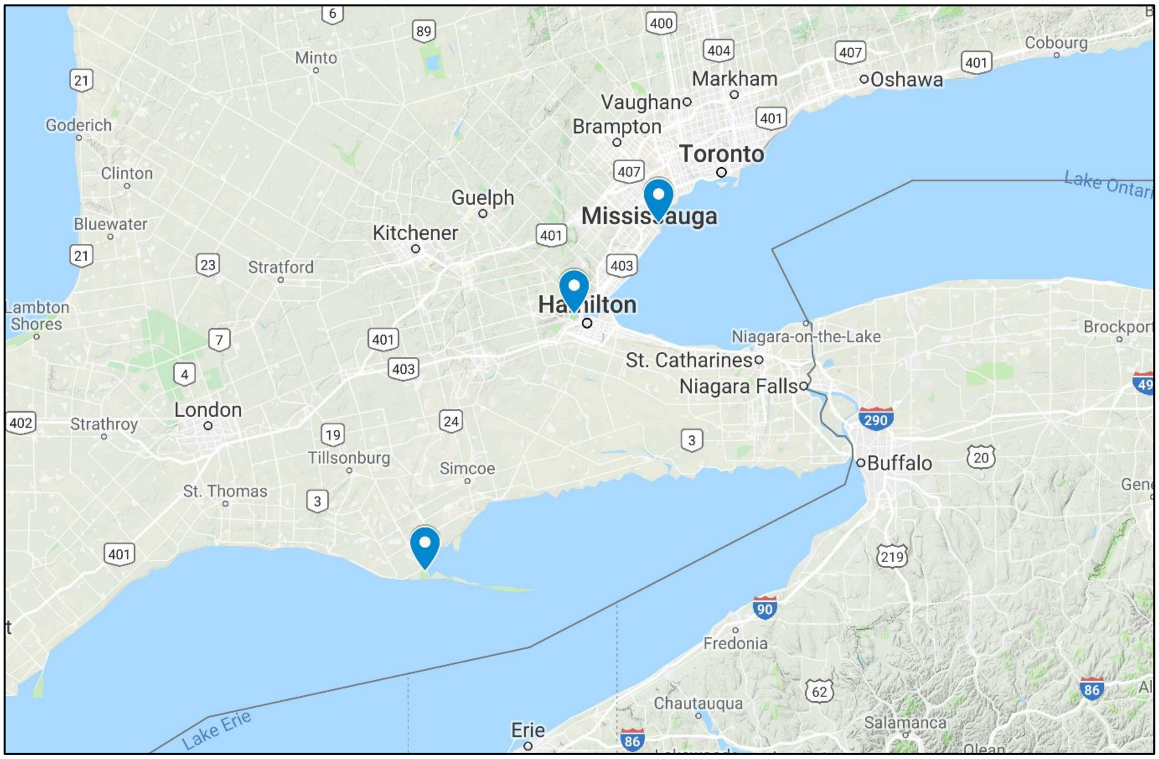


Figure 1: Locations of potential trait sampling sites in Southern Ontario (Google, 2019)

Using the criteria of accessibility, safety, and the need to be able to do a well-designed comparative study of similarly aged wetlands and traits and features that were at similar successional states, I selected the Long Point Crown Marsh, a freshwater, coastal wetland in Long Point (Port Rowan) Ontario (Figures 1,2) managed by the Ontario Ministry of Natural Resources and Forestry (OMNRF). Located on the northern shore of Lake Erie, Long Point is a sand peninsula, and is classified under the United Nations Educational, Scientific and Cultural Organization (UNESCO) as a World Biosphere Reserve, and a Ramsar Convention significant wetland (OMNRF, 2017b; Bolton and Brooks, 2010). Long Point and its wetlands are an especially crucial habitat for migratory wildlife, especially birds (Badzinski et al., 2008; OMNRF, 2017b). It is also home to crucial fish spawning habitat and is used recreationally for a wide range of uses, including hunting, fishing, camping and hiking (Figure 3) (OMNRF,2017b).

Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AeroGRID, IGN, and the GIS User Community

Figure 2: The Crown Marsh, with created open water channels and ponds in blue; sampling sites identified in red

Figure 3: Partnership sign at the entrance to the Crown Marsh, signifying a variety of uses for the wetland complex

Coastal wetlands in the Great Lakes are characterized by water level fluctuations that facilitate the development of emergent vegetation (Figures 4,5) during periods of low water levels, and vegetation that can tolerate periods of higher water levels (Tulbure et al., 2007). Coastal wetland threats include drainage, stabilizing water levels and sedimentation (Schummer et al. 2012). They are also threatened by invasive species establishment, whose invasion is facilitated by anthropogenic influences (Tulbure and Johnston, 2010). While open water is beneficial for biodiversity, it is common for Great Lakes wetlands to be invaded by cattails (*Typha x glauca, Typha. angustifolia)*, or the non-native *Phragmites australis* (Cav). Trin. Ex Steud. (hereafter phragmites; common name is 'European common reed') (Badzinski et al., 2006). Phragmites is an emergent plant that invades wet marshes, lake edges, roadsides, woodlands and also rocky terrain (Mal & Narine, 2010). It reproduces by perennial rhizomes and by seed, forming dense monoculture stands, reducing ecosystem biodiversity (Mal & Narine, 2004; Tulbure & Johnston, 2010). Its ecological advantages over native flora include its rhizome growth rate of 1-2m/year, ability to grow taller, rapid stolon growth, production of more biomass, and ability to shade out native species (Tulbure & Johnston, 2010; Wilcox et al., 2003). While native strains of phragmites (*Phragmites australis subsp. americanus*) have been found in Long Point marshes in the past, non-native phragmites also outcompetes the native strain, in addition to in *Typha spp.*, wetland and marsh meadow communities (Schummer et al., 2012; Wilcox et al. 2003). In a habitat suitability assessment by Carlson Mazur et al. (2014), it was suggested that the lower two Great Lakes, Erie and Ontario, were most susceptible to future Phragmites invasion in the Great Lakes region. In the Crown Marsh, phragmites has invaded and dominated some of the best quality habitat for wetland flora and fauna within the Great Lakes basin (Badzinski et al., 2008). Phragmites has been reported in Ontario as early as 1874, and in the Long Point region since 1945, but began to exponentially increase in abundance in the late 1990s (Badzinski et al. 2008; Mal & Narine 2004). Between 1995-1999, phragmites increased by 50% each year due to mild temperatures, low Lake Erie water levels and the establishment of the non-native strain of phragmites (Badzinski et al., 2008; Wilcox et al. 2003). It has been speculated that the coastal marshes in the Long Point region are nearing an ecological state that will deteriorate further and perhaps irreparably unless phragmites is controlled (OMNRF, 2017b).

Figure 4: Typical emergent vegetation in the Crown Marsh; native species seen here, mixed with *P. australis*

In response to ‘Ontario’s worst invasive plant’ (Catling and Mitrow, 2005) aggressively invading the Crown Marsh, the OMNRF, in partnership with the Long Point Waterfowler’s Association (LPWA), a local hunting organization, began restoration work in the Crown Marsh in 2006 by way of creating dug ponds for increased open water area (LPWA, 2017). It is worth noting that pond excavation and aquatic vegetation removal has occurred periodically since the 1960s, but substantial work has been since 2006 (Badzinski et al, 2007). Dry, over-vegetated areas of the marsh have been excavated to create ponds of varying depth and size (Figure 5). The restoration work was conducted with a goal to improve marsh habitat by increasing open water areas, as well as overall flow and connectivity within the marsh (predominantly for waterfowl). An assessment by Schummer et al. (2012) found that the creation of open water ponds in the Crown marsh increased plant species richness as well as marsh bird relative abundance and macroinvertebrates. As of January 2017, the LPWA has added 142 acres of open water to the Crown Marsh (Figure 6), the majority of which was completed after 2012 (LPWA, 2017, Cleland, 2016).

Figure 5: Typical emergent vegetation (early spring) on the perimeter of created open water ponds in the Crown Marsh

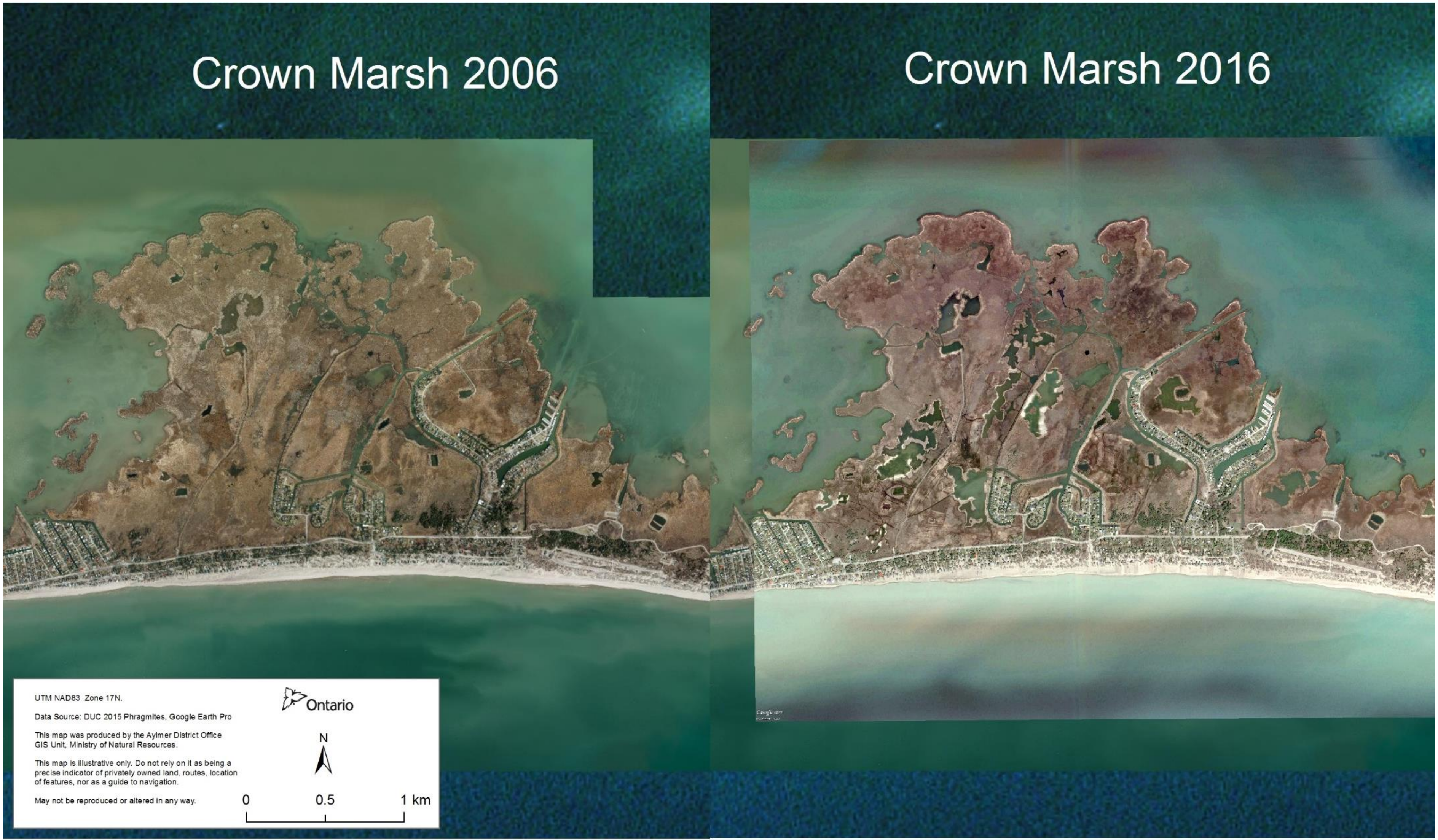


Figure 6: Changes in the Crown Marsh open water between 2006 and 2016, through open water pond creation (MNRF, 2017b, used with permission)

In order to further the improvement of biodiversity and ecological integrity of the Crown Marsh, the Ontario Ministry of Natural Resources, in partnership with the Nature Conservancy of Canada (NCC), has also been conducting intensive phragmites management in the Crown Marsh since 2014 (OMNRF, 2017b). Dry areas with phragmites have been treated by ground spraying of glyphosate since this time; aerial spray by helicopter was added to their treatment regime in the fall of 2016 (Cleland, 2016, OMNRF, 2017b). An emergency registration permit was granted in 2016 by Health Canada's Pest Management and Regulatory Agency, to allow wet areas to be sprayed with glyphosate-active herbicide (Roundup® Custom for Aquatic & Terrestrial Use Liquid Herbicide, Registration Number 32356 Pest Control Products Act), by aerial and ground application (OMNRF, 2017b). The herbicide used in these areas is permitted for use in the United States, and is commonly used for wetland restoration work (OMNRF, 2017b). In 2017, follow up ground spraying occurred at sites that were previously covered by aerial spray in 2016. Herbicide in general is a popular control method for phragmites; a survey conducted by Martin & Blossey (2013) found that from 2005-2009, 94% of phragmites management projects used herbicide as a control method.

The response of wetland biota (e.g. amphibians, fish, aquatic invertebrates) to glyphosate application varies. It has been reported that glyphosate or its associated surfactant (added to the herbicide to increase its ability to penetrate leaves) has negatively impacted species of arthropods, but had no effect on zooplankton, aquatic insects in snails in other studies (Kulesza et al., 2008). A risk assessment study by Solomon et al. (2003) concluded that while glyphosate was thought to be low risk to aquatic biota, associated surfactants were less understood, which has been supported in other phragmites management reviews (e.g. Hazelton et al., 2016). Glyphosate exposure has also been shown to cause stress in turtles (Hertier et al., 2017), but impacts on amphibians (e.g. anurans) can be species specific and vary by life cycle stage (Govindarajulu, 2008, Gruber et al., 2008, Relyea, 2005). Fish responses to glyphosate also vary by life stage; while adult fishes may not necessarily experience adverse effects from glyphosate contact, young of the year individuals may be negatively affected (Folmar et al., 1979). As a result of the potential risk that glyphosate could pose to aquatic biota, the OMNRF (2017b) is monitoring water quality, invertebrates, fish and fish habitat as part of their follow-up monitoring to herbicide application, in addition to the vegetation community.

In addition to herbicide application, the OMNRF (2017b) is managing the marsh using an integrative pest management approach, which includes other management techniques such as burning, rolling and cutting. Mowing or cutting, which involves the manual removal of stems and seed heads, does not cause an overall reduction in phragmites density if applied as a single management technique (OMNRF, 2011). Cutting and mowing alone can increase the density of phragmites shoots, though it does reduce height and biomass (Hazelton et al., 2016). This is because cutting does not impact the below ground root and rhizome system (OMNRF, 2011). Mowing is usually best in monoculture sites, to avoid damaging native biota (OMNRF, 2011). Cutting in conjunction with high water levels or flooding can assist with control (Hazelton et al. 2016). Similarly, burning is only effective in conjunction with another removal technique, such as herbicide application (Hazelton et al., 2016). In an integrative management context, mowing, cutting and rolling are beneficial because this causes biomass compaction and creates better conditions for a prescribed burn, which is used to reduce biomass (OMNRF, 2011). It is beneficial to treat with herbicide prior to mowing or cutting, because this reduces moisture in the plant (OMNRF, 2011). Burning, however, is not effective without prior herbicide application (OMNRF, 2011). Pending the availability of resources and environmental conditions in the Crown Marsh, the OMNRF (2017b) intends to cut or roll areas within 3-4 weeks of herbicide treatment, with a prescribed burn to follow.

2.2 Trait Selection

Trait information was derived largely from selection and measurement protocols developed by Lavorel et al. (2007), Cornelissen et al. (2003) and Perez-Harguindeguy et al. (2013). Traits were selected as candidates to be used within the Crown Marsh based on two guidelines:

1. Is there potential for a given trait to respond to the restoration-driven disturbances in the Crown Marsh (e.g. vegetation community manipulation through phragmites control) (Funk et al., 2008)?
2. Practical Relevance: Are the trait collection protocols realistic: can they be collected by one or two individuals? Can a given trait be measured in a time efficient and cost effective manner?

Using these two guidelines, a preliminary short list of seven traits was selected, which included whole plant traits, leaf traits and regenerative traits (Lavorel et al., 2008) (Table 1). Below ground traits were omitted as they have been cited as difficult traits to measure (Lavorel et al. 2007). Soft traits were chosen to represent different response traits that would respond to the restoration work being completed in the Crown Marsh (D'Astous et al., 2013; Engst et al., 2016). Whole plant traits, leaf traits and regenerative traits were selected as they all represent key strategies needed to combat invasive species invasions, a disturbance that is widely present in Great Lakes coastal wetlands. It has been suggested that seed mass, plant height and relative growth rate (to which specific leaf area has been suggested as a proxy measure) are key traits to include in trait-based studies, especially when measuring competitive ability in a plant community (Perez-Harguindeguy et al., 2013; Weiher et al. 1999). These three traits have also been supported by those identifying traits along plant ecological strategies (Westoby 1998), as well as research seeking to identify traits that will respond to disturbance regimes and environmental change (Weiher et al. 1999).

Table 1: Short-list of potential traits that could be measured in the Crown Marsh

Trait Category	Functional Trait	Description	Relevant Proxy Measure:	Sources
Whole Plant Trait	**Plant height**	Distance from soil to top of photosynthetic leaves	Response to disturbance, competitive strength, plant defense, effects on disturbance regime	Lavorel et al. 2007, Cornelissen et al. 2003, Egnst et al. 2016, Kyle and Leishman 2009, Buyn et al. 2013
Whole Plant Trait	**Spinescence**	Presence of spines	Competitive strength, plant defense/protection, herbivory	Lavorel et al. 2007
Leaf Trait	**Specific Leaf Area (SLA)**	Area of leaf divided by oven-dry mass.	Competetive strength, plant defense/protection, shade presence/sun limitation	Funk et al. 2008, Kunstler et al. 2016, Garnier et al. 2003, Lavorel et al. 2007, Egnst et al. 2016, Kyle and Leishman 2009, Buyn et al. 2013
Leaf Trait	**Leaf Dry Matter Content (LDMC)**	Oven dry mass divided by water saturated fresh mass	Plant defense/protection, effects on disturbance regime	Lavorel et al. 2007, Garnier et al. 2003, Egnst et al. 2016, Kyle and Leishman 2009, Buyn et al. 2013
Regenerative Trait	**Dispersal mode**	A categorical trait – how does a plant disperse (e.g. seed, fruit, spore)?	Response to disturbance	Lavorel et al. 2007, Cornelissen et al. 2003
Regenerative Trait	**Dispersule shape and size**	Size is the mass of a seed plus all additional dispersal parts; shape is variance of the length, width and thickness of dispersule unit	Response to disturbance	Lavorel et al. 2007, Cornelissen et al. 2003
Regenerative Trait	**Seed mass**	Oven dry mass of a seed	Response to disturbance, competetive strength, plant defense/protection, shade presence/sun limitation, fecundity	Lavorel et al. 2007, Egnst et al. 2016, Kyle and Leishman 2009, Buyn et al. 2013, Weiher et al. 1999

Specific leaf area (SLA) and leaf dry matter content (LDMC) were the two traits selected for this study, as they were the easiest to measure given resource and time constraints. I arrived at these choices via processes of elimination. Before field research began, I had removed spinescence from my choices of measurable traits. While an easy trait to measure, it would be unlikely that it would directly respond to the restoration work completed in the marsh. Dispersule size and shape were also ruled out at this time, in favour of regenerative traits that could be measured on their own (e.g. seed mass). Plant height was eliminated because it would need to be measured at the same time, for all plots – this was not logistically feasible for one person. Seeds were collected and data collated through the field study but ultimately were eliminated from analysis because the vegetation at the sites had few seeds.

2.2.1 Specific Leaf Area

Specific leaf area (SLA) is defined as the area of one side of a leaf, divided by its dry-mass, expressed in m^2kg^{-1} (Cornelissen et al., 2003; Pérez-Harguindeguy et al., 2013). Specific leaf area has been considered within a suite of traits to measure that capture a key component of plant life cycles (Engst et al., 2016; Pérez-Harguindeguy et al., 2013). It is also a common trait proposed for key plant life cycle strategies, such as the leaf-height-seed strategy proposed by Weiher (1999). Specific leaf area incorporates the cost to produce a leaf (mass) against the benefits that a leaf provides in terms of photosynthetic area (Vernescu and Ryser 2009). This cost and benefit are also connected; the larger the leaf, the more structures required to keep the leaf supported, and the larger the mass (Vernescu & Ryser, 2009). It has been used in literature as a 'soft trait' for many additional, harder to identify, functional characteristics, such as relative growth rate, photosynthesis per leaf dry mass, leaf nitrogen and leaf lifespan (Cornelissen et al., 2003; Gondard et al., 2003; Poorter et al., 2009; Weiher et al., 1999).

In general, areas that are wet, or have high resource availability (e.g. nutrients) have larger specific leaf area (Cornelissen et al., 2003; Lavorel et al., 2007; McCoy-Sulentic et al., 2017). Specific leaf area has also been found to represent a plant's competitive ability, since quick growth is crucial if a plant is to compete with other organisms in a community (Engst et al., 2016; Kunstler et al., 2016; Weiher et al., 1999). Specific leaf area also varies significantly with sunlight availability; leaves in sun have a thicker lamina and their specific leaf area is lower than leaves in the shade, who have specific leaf area values that can be twice that of sun-exposed leaves, even on the same plant (Hodgson et al., 2011). In addition to the ecological responses that can be inferred from specific leaf area, it has been hailed as a relatively easy trait to measure (Garnier et al., 2004; Hodgson et al., 2011).

2.2.2 Leaf Dry Matter Content

Leaf Dry Matter Content (LDMC) is measured by dividing a leaf's oven dry mass by its water-saturated fresh mass, expressed in mg g^{-1} (Pérez-Harguindeguy et al., 2013). Also hailed as a relatively easy trait to measure, leaf dry matter content responds to both competitive ability and soil nutrient availability (Engst et al., 2016; Garnier et al., 2004; Lavorel et al., 2007). Leaves that have high leaf dry matter content are tougher and therefore thought to be better suited to deal with stressors like herbivory, wind and weather hazards (Cornelissen et al., 2003). Low leaf dry matter content leaves typically signify productive and disturbed environments (Cornelissen et al., 2003). There is also a strong relationship between specific leaf area and leaf dry matter content (Garnier et al., 2001). High specific leaf area and low leaf dry matter content suggest that a plant is producing a significant amount of biomass (Garnier et al., 2001). Conversely, low specific leaf area and high leaf dry matter content suggests an attempt to retain nutrients (Garnier et al., 2001).

2.2.3 Use of Specific Leaf Area vs. Leaf Dry Matter Content

Both traits have potential to explain similar trait-environment responses, but sometimes they differ (Cornelissen et al., 2003; Hodgson et al., 2011). Specific leaf area generally correlates better than leaf dry matter content trait values with relative growth rate (Cornelissen et al., 2003). Leaf dry matter content has been proposed as a better trait for interpreting cases of soil fertility (Hodgson et al., 2011). Practically, leaf dry matter content could prove to be a better leaf trait to measure in cases where organisms do not have planar leaves, or leaf area measurements are difficult to obtain (Cornelissen et al., 2003; Hodgson et al., 2011). For practical reasons, leaf dry matter content was chosen as an additional leaf trait for the simple reason that wet weight could be quickly and easily calculated at the time of specific leaf area collection, as the same leaf can be used for both specific leaf area and leaf dry matter content calculations (Cornelissen et al., 2003; Pérez-Harguindeguy et al., 2013).

2.3 Data Collection

2.3.1 Sampling Protocol

Within the Crown Marsh, there are approximately 15 created pond clusters (Veenhof, 2017a, 2017b). In 2017, from early April to Early June, five site visits were conducted to select ponds to sample. Ponds were selected based on accessibility; if they were only accessible by boat or canoe, they were eliminated. If they had little to no vegetation growing around the perimeter (e.g. herbicide treatment had been too recent to allow for natural regeneration), they were also eliminated. Three ponds were then selected: Malcolm, Ankney and a cluster of three ponds created for Fowler's toads (*Anaxyrus fowleri)*, hereafter referred to as Fowler's Ponds (Figure 7). The former two ponds were named in honor of prominent waterfowl conservationists in the Long Point Region, and ranged from 2 (Fowler's Ponds), 4 (Malcolm), and 6 (Ankney) years of age. These particular ponds were selected due to their accessibility and the range of pond ages that was available to sample.

Approximate pond areas were calculated in Google Earth using satellite imagery from April 2016. A target of 3-5% of the perimeter was set, based on the estimate that it could take up to half an hour per plot. This target was also set with a secondary assumption that leaf collection days could not be a full 8 hours, in order to prevent samples from being compromised by heat or transport. Due to the size of Malcolm and Ankney ponds and timing constraints within other parts of the research process (e.g. transport and lab time, collection time in the field), only the southern parts of each pond could be sampled. Twenty four to twenty six plots were selected per pond complex to get a sense for trait variability with the age of each pond (Figure 7). An additional 25 plots were also sampled in the open water marsh (OM).

Figure 7: Approximate locations of ponds and sampling plots in the Crown Marsh

1m^2 quadrats were used (identified by flags & GPS coordinates) to sample continuous emergent vegetation around open water perimeter of all dug ponds (Figure 8). This was chosen as opposed to more traditional straight transect methods, as the impact of the restorative effort (e.g. pond creation and phragmites control) on emergent vegetation was the target of this study and would be the highest around the pond perimeter. Given the magnitude of the restored area of the Crown Marsh, the ponds acted as an easily identified component of restoration work that could be assessed.

Quadrats were spaced evenly around the pond perimeter (every 30 meters). In three cases, obstacles (e.g. bird nests, fallen trees) prevented this 30m spacing; in this case, the plot was situated 20 meter from the previous plot. If there was no emergent vegetation at the assigned area for sampling, ‘no vegetation’ was noted and sampling continued at the next quadrat. If the vegetation was not healthy enough to sample within a quadrat and no other healthy plants were present within a 5-meter radius of the plot, the quadrat was not sampled for traits, but species richness data was recorded. This occurred in two cases. If a plot was adjacent to a spoil pile from a pond’s creation, the bottom of each 1m^2 quadrat was placed at the edge of the pond spoil pile, and extended into the water to sample continuous emergent vegetation. If the plot was not located on a spoil pile, quadrats were situated where continuous emergent vegetation began.

Figure 8: Examples of sampling quadrats in the Crown Marsh (Ankney Pond)

Emergent vegetation was classified in this study as any species that stood erect out from the perimeter bank or shallow bottom of the perimeter of each pond with the majority of their leaves, stems or flowering structures above water.

Floating emergents were excluded from trait analysis. This decision was made because not only were most submergent plant structures located below water, but they were almost never the dominant species in research plots (occurred in 3 plots). In the select few plots that they were dominant, it would have been impossible to identify individual plants without a researcher submerging themselves under water to find root systems. Because of the difference in structure and support in aquatic plants, in comparison to terrestrial plants, the way in which specific leaf area should be interpreted is currently unknown (Catford & Jansson, 2014).

Trait data were collected for the top 1-4 species in each quadrat. All species were identified, with species abundance calculated using percent cover, up to 100%. This 100% threshold was selected over percent covers greater than 100 as the emergent vegetation did not have clear division of a canopy, commonly seen in forest or grassland studies (ground cover vs. mid canopy vs. dominant canopy). Percent cover was chosen over other measures of abundance, such as biomass, as this is the most rapid way to assess abundance (Lavorel et al. 2008). Plants were identified to species where possible, using *A Field Manual of Michigan Flora, Wetland Plants of Ontario, and Newcomb's Wildflower Guide*. Initial species ID was conducted at the first visit to each plot; ID verification was also conducted at the time of seed sampling, and through pressed specimens at the end of the season.

2.3.2 Leaf Trait Collection

Two leaves from 5 individuals, or 10 leaves from 10 individuals (depending on the number of leaves on each plant and the type of species) were sampled from the 1-4 most dominant species in each quadrat; this was consistent with previous protocols (Cornelissen et al., 2003; Pérez-Harguindeguy et al., 2013). Ten to fifteen plants were always collected in case samples did not survive transport. Plants were sampled 2-3 hours after sunrise and 3-4 hours before sunset (Garnier et al., 2001). If there were an insufficient number of plants to sample within the quadrat, samples were taken from the next nearest neighbouring plants, up to a 5-meter radius around the m^2 quadrat. Whole plant stems were cut to obtain these leaf samples, to maximize the amount of water retained in the leaves (Pérez-Harguindeguy et al., 2013). Average sized leaves from average sized plants for the quadrat or area were taken from plants to provide the best estimation of leaf area. For species with small basal leaves that were often absent when submerged in water for long periods of time (e.g. *Schoenoplectus spp., Eliocharis spp. Equistem spp.)*, a 'functional analogue' of the leaf was chosen to standardize collection

(Cornelissen et al., 2003). In my case, the functional analogue was a 10cm length of the middle of each specimen, as this would act as photosynthetic tissue.

Upon collection, samples were immediately placed in plastic bags with a damp paper towel around the cut portion of the stem, and during transport back to the lab they were held in a cooler with the base of the stem placed on ice to minimize exposure to a water-unsaturated environment (Garnier et al., 2001; McCoy-Sulentic et al., 2017; Ryser et al., 2008). Whole stems were immediately placed in beakers with distilled water in lab, and placed in the fridge overnight at low temperatures to reduce the risk of rotting (Garnier et al., 2001; Pérez-Harguindeguy et al., 2013). Fridge temperatures of 2-6°C are suggested by Perez-Harguindeguy et al. (2013); fridge temperatures could not be stabilized, but were kept at the lowest setting possible, which was approximately 5°C.

After rehydration, leaves were removed from whole stems. Samples were weighed (to three decimal places) to record wet weight for leaf dry matter content calculations. Due to the small size of most leaves, the scale was calibrated every 10 samples to increase measurement accuracy. Each leaf was scanned against a white background on a Canon CanoScan LiDE 220 colour scanner at 600dpi. Each leaf was pressed against the scanner using a 2mm glass plate to standardize each leaf area and to ensure that leaves were as flat as possible; the weight exerted by the glass is considered minor (Figure 9) (Vernescu & Ryser, 2009). After weighing, samples were placed in a drying oven at either 70 degrees for a minimum of 60 hours, or 80 degrees for a minimum of 48 hours, in order to ensure all moisture was removed (Cornelissen et al., 2003). Dry weight was recorded as soon as samples were removed from the drying oven, to ensure that dried leaves did not take up moisture from the air (Cornelissen et al., 2003).

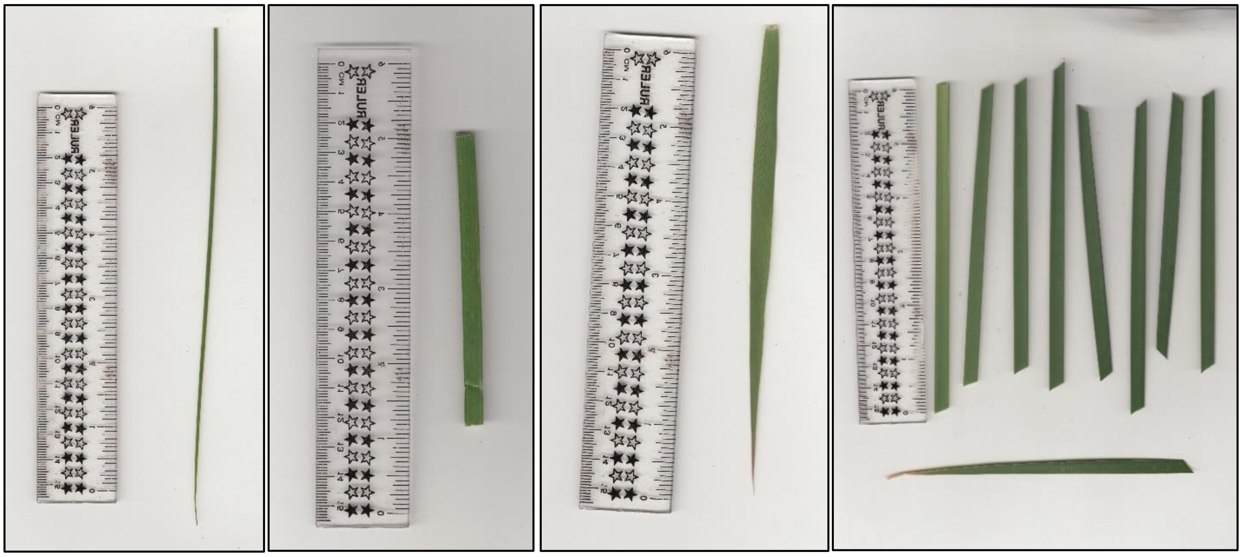


Figure 9: Area scans of sampled leaves

2.3.3 Data Collection Timing and Effort

Trait sampling for specific leaf area and leaf dry matter content, as well as species abundance data, were collected when peak standing biomass would be the greatest in the Crown Marsh (Pérez-Harguindeguy et al., 2013). Sampling took place from June 21st to September 23rd, 2017, and a total of 17 trips were taken to the Crown Marsh in 2017.

Because wetland plant communities can take up to 20 years to recover after restoration (Stefanik & Mitsch, 2012), the study was designed with the expectation that any changes in plant trait values would be negligible over the course of even 2-3 years. In 2017, trait data were collected, for specific leaf area and leaf dry matter content, for the top 1-3 dominant species in each quadrat plot. This protocol was selected in acknowledgement of the mass ratio hypothesis (Grime, 1998), which suggests that the most dominant species contribute the most to a community. It has been proposed that a minimum of 80% of the standing biomass (in the case of this study, percent cover was used as a measure of abundance) in a community should be sampled in trait-based studies (Cornelissen et al., 2003; Pakeman & Quested, 2007; Pérez-Harguindeguy et al., 2013; Wright et al., 2004). This is in contrast to evolutionary focused studies, where species would likely be chosen to represent phylogenetic diversity, as opposed to biomass targets (Pérez-Harguindeguy et al., 2013). The top 1-3 dominant species per plot did not meet this 80% threshold in every sampled plot, which warranted four additional visits in June and July of 2018, during the months of June and July, to collect additional species for both trait datasets. Three plots, as a result, had leaves collected for four species.

2.4 Data Collation and Management

2.4.1 Raw Trait Data

A total of 22 quadrats was included in the analysis for the Fowler's Pond (FP) complex, 23 for Malcolm Pond (MC), 24 for Ankney (AK) and 25 for the open marsh (OM) plots. This quadrat reduction occurred because 6 plots (2 Frog Pond, 3 Malcolm, 1 Ankney) had no plants at the time of sampling.

Prior to conducting any non-parametric tests, it was confirmed that the 2018 data were not outliers within each treatment group through visual comparison of boxplots and the raw dataset. In addition to visual examination, if any plots were treated for phragmites in 2017, between the two seasons of data collection, these data were omitted to ensure that all data points remained consistent regarding phragmites treatment. Five samples from four plots were excluded from the 2018 data set for this reason, for a total of 50 leaves. It was accepted that these plots would not achieve the 80% sampling target. Forty leaves were also excluded from the 2017 data set due to data entry errors. While only five leaves were compromised of the 40 leaves excluded, all data points for each species were omitted to remain consistent with sampling protocols of 10 leaves per species, per quadrat. As a result, 1670 leaves in 2017 and 280 leaves in 2018 were included in the statistical analysis.

2.4.2 Leaf Area Calculations

Leaf images were processed through the statistical software R (V. 3.4.2), using the package LeafArea (Katabuchi, 2015). This allowed for batch processing of images to calculate leaf area, using the software ImageJ (Rasband, 1997), in a time efficient manner. The R script for this process can be found in Appendix I. In some cases, LeafArea was unable to identify leaf area in the scanned image. In this case, leaf area was manually determined using ImageJ. This occurred in two cases for *Schoenoplectus pungens* (Common Three Square Sedge). The majority of *Typha spp.* were not correctly identified by Leaf Area. For this reason*, all Typha spp.* were calculated manually using imageJ, without automated processing in R, to allow for area calculation methodology to remain consistent for the species.

2.4.3 Community Weighted Mean

As proposed by Garner et al. (2004), community weighted mean (CWM) involves weighting the average of trait values by their relative abundances (Ricotta & Moretti, 2011). Community weighted mean has been proposed from the mass ratio hypothesis; originally explained by Grime (1998), the mass ratio hypothesis suggests that if a species makes up a dominant part of the standing biomass in a community, then it is more likely that their traits will have more of an influence on ecosystem properties than less dominant species (Grime, 1998; Pakeman & Quested, 2007; Ricotta & Moretti, 2011). Used in many trait-based studies, community weighted mean quantifies the dominant traits values in a community, and can explain the changes in mean trait values in plant communities with environmental changes (Ricotta & Moretti, 2011). Community weighted mean is relatively robust to changes in methodology when measuring species abundances, which makes it a good method for comparative studies where monitoring efforts may differ (Lavorel et al., 2008; Garnier et al., 2016, p. 99). Community weighted mean was calculated using the following formula:

$$\mathrm{CWM} = \sum_{i=1}^{S} p_i x_i$$

Where p_i is the mean value of a trait, i is the relative abundance of a given species in the community and x_i is the trait value of a given species. Community weighted mean was calculated for each plot in each pond and open marsh complex (Ricotta et al., 2015).

2.5 Statistical Analysis

Leaf dry matter content, specific leaf area and community weighted mean were all calculated using Microsoft Excel 2015, and all subsequent statistical analysis was done using R (V.3.4.2, Appendix II). Plots were divided by two treatments; community weighted means for both traits were compared between pond, as well as whether they were treated by glyphosate. Using aerial maps provided by the OMNRF, (hand drawn and created using GIS software), each plot was classified as having had herbicide treatment in 2016 or not (treatment = yes, no). Rolling and cutting management only occurred in approximately 10-12 Fowler's Pond plots and 2-4 Ankney plots, so were not included as a treatment in the analysis. Specific leaf area and leaf dry matter content were analyzed separately in all cases so data remained independent, since both traits were measured from the same leaf samples.

Community weighted mean values were calculated per plot, for both specific leaf area and leaf dry matter content; in other words, one data point was calculated for each trait, per plot (Muscarella & Uriarte, 2016). This particular dataset has an unbalanced design (22, 23, 24, and 25 samples in each pond, and 67 and 27 for treated and untreated by herbicide). While one can use unbalanced designs in parametric analyses, the risk of violating the equal variance assumption is higher with unbalanced designs, which was taken into consideration when parametric assumptions were tested (Decoster, 2006).

Normality assumptions were tested using the Shapiro-Wilk normality test (p = 0.05). The Shapiro-Wilk was chosen because it has been found to have the best power when looking at asymmetrical distributions (Yap & Sim, 2011). Since the distribution of the trait data for the Crown Marsh is unknown, the Shapiro-Wilk was also selected to be the most conservative approach. A Levene's test for equality of variance was then run, comparing the absolute deviations of each observation against its group median, as opposed to mean. This is proposed to be the more robust comparison; the use of the group median is the default method in R (V.3.4.2). A Levene's test was chosen as the dominant test for equal variances in this study, over other tests such as Bartlett’s as the Levene’s test is less sensitive to normality departures, which were common in the trait datasets.

2.5.1 Comparison between Ponds

When the assumptions of normality were violated, a non-parametric Kruskal Wallis test was used (p=0.05), with a threshold of p = 0.05. While non-parametric approaches are not often recommended by statisticians for ecological data as they are less powerful than parametric equivalents, non-parametric tests are robust to measurement error (Gotalli & Elliosn, 2013, p.121). As all datasets met the assumption of equal variance, a Kruskal Wallis was chosen as a conservative approach. A Dunn’s Kruskal Wallis Multiple Comparisons Post Hoc test was conducted to determine differences in medians. A Dunn test has also been found to be useful for groups with unequal number of observations (Zar 2010, Mangiafico, 2015).

2.5.2 Comparison between Herbicide Treatment Groups

If parametric assumptions were met, a Welch’s t-test was selected to run to compare herbicide treatment groups (treatment = yes, no). This is opposed to Student’s t-test, as a Welch’s t-test is better suited for data that have unequal sample sizes (Delacre et al. 2017). However, normality assumptions were violated for both datasets, so a non-parametric Mann-Whitney U (also known as a Wilcoxon Rank Sum) test was chosen (Mann & Whitney, 1947), which tests whether a random sample taken from two populations will be less than or greater than each other, and does not require the assumption of normality.

2.6 Results

A total of 20 taxonomic groups were sampled for traits over the 94 sampling plots, with Equistem spp., Eliocharis spp. and Typha spp. identified at the genus level (Table 2).

Table 2: Species sampled for traits in the Crown Marsh

Carex viridula	*Juncus spp. (unknown)*	*Prosperpinasa palustris*
Carex pellita	*Juncus spp.* 2 *(unknown)*	*Rosa blanda*
Eliocharis spp.	*Lycopus americanus*	*Schoenoplectus pungens*
Equistem spp.	*Lycopus uniflorus*	*Schoenoplectus tabernaemontani*
Eupatorium perfoliatum	*Lysmachia thyrsiflora*	*Typha spp.*
Juncus articulatus	*Phragmites australis*	*Unknown grass spp.*
Juncus effusus	*Populus deltoides*	

Overall, the dataset represented 83.4% of the cover identified in the 94 plots. When broken up by treatment, only Malcolmn Pond, and the plots that did not receive herbicide, fell short of the 80% target identified in literature (Table 3) (Cornelissen et al., 2003; Pakeman & Quested, 2007; Pérez-Harguindeguy et al., 2013; Wright et al., 2004).

Table 3: Treatment groups that met the 80% sampling target in the Crown Marsh. Numbers in each row represent the percent of plots, per treatment, that reached each sampling target. For example, 80% of plots sampled in the Open Marsh (OM) had 80-100% of the plant community sampled for traits

		Treatments (%)					
		Pond				**Herbicide**	
		AK	**FP**	**MC**	**OM**	**Yes**	**No**
% Vegetation Sampled	**0-39%**	16.67	0	4.35	0	1.49	14.81
	40-79%	29.17	0	26.09	20	13.43	22.22
	80-100%	54.17	100	69.56	80	85.07	62.96
Number of Plots per Treatment (n)		24	22	23	25	67	27

Approximately 57 hours was spent in the Crown Marsh in 2017 (including travel time between ponds and plots), with an additional 14 hours in 2018; 3.9 hours were spent in the marsh per visit. 7-10 plots were sampled on each initial visit in 2017, with sampling (including species identification, abundance calculation and trait sampling) lasting approximately 30 minutes per plot. Lab processing time for each species (e.g. 10 leaves per species) was approximately 30 minutes for wet weight and 10 minutes for dry weight, though timing did vary by size of the leaf. For example, large leaves such as *Typha spp.* took longer to weigh and scan than smaller leaves like *Lycopus spp.*

2.6.1 Comparison between Ponds

Community weighted mean comparisons between pond and open marsh plots did not yield statistically significant differences for specific leaf area (p>0.05; Table 4, Figure 10). For leaf dry matter content, statistical differences were found between Ankney (AK) pond and the other three ponds.

Table 4: Community Weighted Mean (CWM) results between pond and open marsh plots

Test	Value	LDMC	SLA
Shapiro-Wilk	P-value	0.0013	$2.61\text{x}10^{-10}$
Levene's Test	degrees of freedom	1	1
	F statistic	0.083	0.021
	P-value	0.774	0.886
Kruskal-Wallis	degrees of freedom	3	3
	P-value	0.0019	0.16
Dunn's Kruskal-Wallis Post-Hoc	P-value, unadjusted	AK/FP: 0.0068	N/A
		AK/MC: 0.0059	
		AK/OM:0.0016	
	P-value, adjusted	AK/FP: 0.0041	N/A
		AK/MC: 0.0353	
		AK/OM:0.0098	

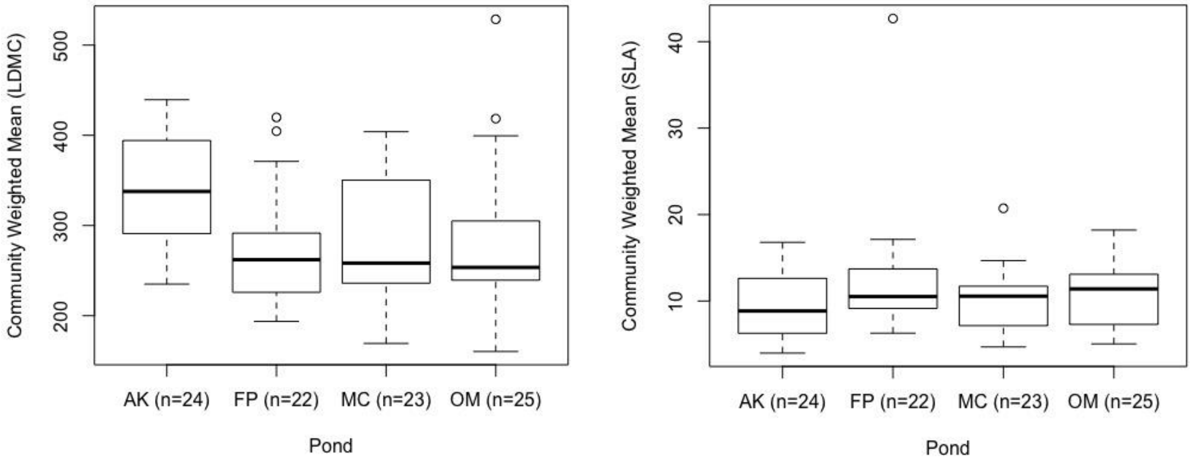


Figure 10: CWM trait comparisons by Pond (AK=Ankney, MC=Malcolmn, FP = Fowler's Ponds, OM= Open Marsh), Community weighted mean for leaf dry matter content (left) is expressed in mg g^{-1} and in m^2kg^{-1} for specific leaf area (right).

2.6.2 Comparison between Herbicide Treatment Groups

Regarding community weighted mean data, the Mann Whitney test showed significant differences, for both traits, between plots that had been treated for herbicide, versus plots that had not been treated in 2016 ($p<0.05$, Table 5). Plots that had herbicide application in 2016 yielded specific leaf area community weighted means higher than those that were not treated; leaf dry matter content community weighted means were lower in plots with herbicide application (Figure 11).

Table 5: Community weighted mean results between herbicide groups

Test	Value	LDMC	SLA
Shapiro-Wilk	P-value	0.0013	2.61x10-10
Levene's Test	degrees of freedom	1	1
	F statistic	0.083	0.021
	P-value	0.774	0.886
Mann-Whitney U	P-value	0.0324	0.005

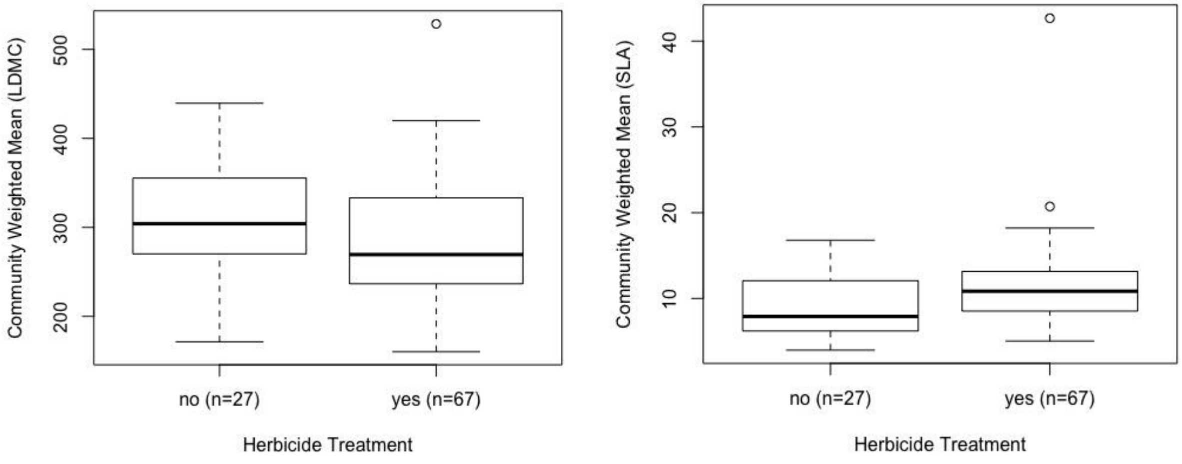
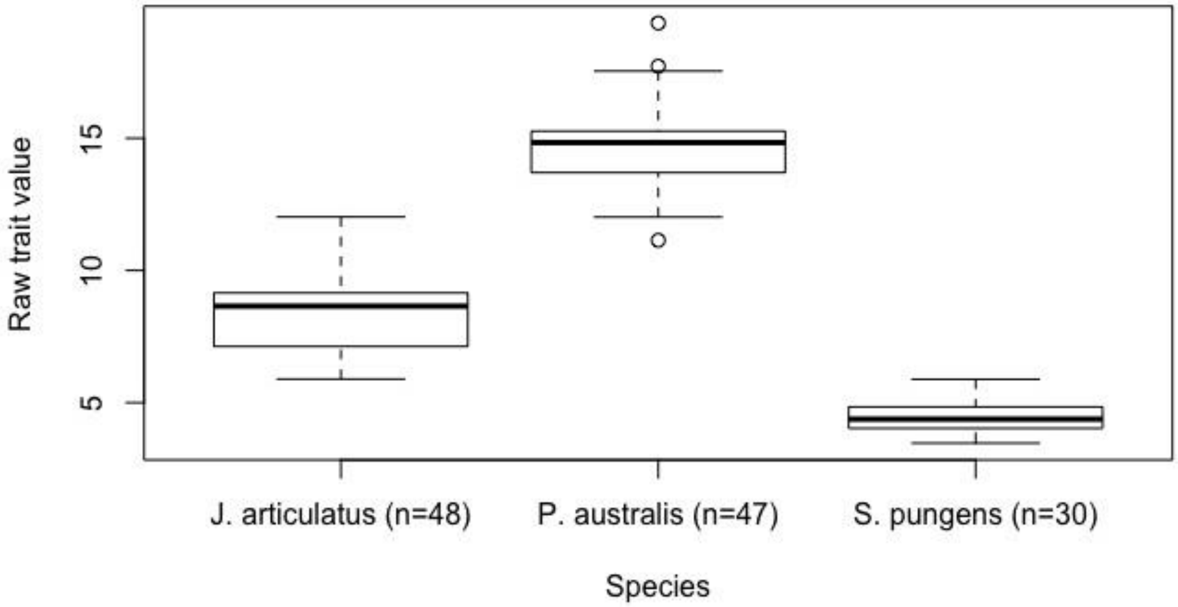


Figure 11: Community weighted mean changes with herbicide treatment, expressed in mg g^{-1} for leaf dry matter content (left) and in m^2kg^{-1} for specific leaf area (right)

2.6.3 Raw Trait Data

Trait data for the top three species, irrespective of their sampling locations and treatments, were also examined, in order to get a descriptive understanding of trait values in the Crown Marsh (Figure 12).



<u>Figure 12</u>: Specific leaf area (in mg^{2}kg^{-1}) trait values for the top three species in the Crown Marsh.

2.7 Discussion

2.7.1 Traits Were Significantly Different After Herbicide Treatment

Community weighted means for both traits did show changes after herbicide treatment. Specific leaf area increased overall with herbicide treatment, and leaf dry matter content decreased (Figure 11). Both specific leaf area and leaf dry matter content are related to plant tradeoffs of producing biomass (resulting in a high specific leaf area and low leaf dry matter content) versus conserving nutrients (resulting in a low specific leaf area and high leaf dry matter content) (Garnier et al., 2001; Li et al., 2017). The patterns exhibited by community weighted mean (CWM) between plots treated for herbicide suggest that traits that promote biomass production may be favoured in response to selection pressure. This is consistent with the notion that herbicide application is designed to decrease plant biomass (targeting areas with high densities of exotic species) during the season of application. Subsequently, there would be an expected increase in biomass production in the season after herbicide application as the plant community regenerates (assuming the application was not repeated then – as was the case in my study site).

There will be a need for more direct tests of herbicide application on plant traits but there is evidence that other ecological restoration actions do affect community weighted mean values. While studying traits as a success measure for restored grasslands, Sandel et al. (2011) found that, when mowed, the specific leaf area community weighted mean increased at their sites; this was mainly because of an increase in leaves that were less dense. Low density leaves are less costly to produce (Drenovsky & James, 2010; Sandel et al., 2011); this is true if thinning leaves are produced but Sandel et al. did not find that trait had an impact. Species with a high specific leaf area tend to grow faster and have a competitive advantage over species with lower specific leaf area (Drenovsky & James, 2010; Ostertag et al., 2009). Comparatively, this same relationship may explain some of my results, especially if phragmites dominant communities are mowed and burned post- herbicide application, which is a proposed management practice in the Crown Marsh (OMNRF 2017b).

Garnier et al. (2004) examined successional trait responses in old fields, and found that as fields aged, composition moved from (a) fast growing (r-selected) species with high specific leaf areas and low leaf dry matter content to (b) K-selected species with low specific leaf areas and high leaf dry matter content (see also Sandel et al. 2011; Zirbel et al., 2017). Ecological restoration treatments could produce a similar, managed outcome: removing the often r-selected exotic species with high specific leaf areas (e.g.(Drenovsky & James, 2010; Gross et al., 2015; Leishman et al., 2007) and directing and speeding succession to K-selected native species. A direct test of this is needed. In my study, I was only able to gather anecdotal observations; while invasive exotic *Phragmites* does appear to have higher specific leaf areas than the native *Schoenoplectus pungens* or *Juncus articulatus* (Figure 12), this remains a hypothesis, and not a conclusion.

One possible constraint to the utility of using (only) community weighted mean assessment of specific leaf area and leaf dry matter content for examining outcomes of ecological restoration is that does not necessarily differentiate between impacts of native vs. exotic non-native species. For example, the goal of the rehabilitation work in the Crown Marsh is to increase open water for waterfowl, and restore the plant community through reducing *Phragmites australis* (Badzinski, 2007, OMNRF, 2017b). Community weighted mean was used to characterize the dominant plant traits of the overall community, and did not differentiate by invasive or native species, hence it obscures the impact and outcomes of managing phragmites. An increase in community weighted mean with herbicide treatment doesn't tease out whether phragmites has been reduced, or whether native species have increased. While we do not have pre-herbicide application trait data to confirm, it is possible that the herbicide application wasn't effective, and an increase in specific leaf area and decrease in leaf dry matter content was simply because phragmites has continued to encroach on native vegetation, since high specific leaf area is also associated with exotic and invasive species. Alternatively, an increase in community weighted mean could also be positive, and mean that sites treated by herbicide have been colonized by early successional species, which are also known to have high specific leaf area (Dawson et al., 2017). Community weighted mean will need to be accompanied by simple taxonomic monitoring to confirm a reduction in invasive species (i.e. invasive spread is not the reason for an increase in specific leaf area). The measurement of invasive species cover is already a proposed component of wetland vegetation monitoring protocols (Albert, 2008; Lawson, 2004).

Overall, the comparison of traits between management regimes shows promise for the future of trait use for restoration work. If management conditions can be controlled, it is possible to monitor changes in community trait values over time. However, the complexity of Phragmites management will play a key role in the ability to utilize traits in an adaptive monitoring protocol for the Crown Marsh. Control methods for Phragmites require multi-year application in order to be effective, making successful management challenging (Carlson Mazur et al., 2014). The ability of the OMNRF to treat Phragmites in follow-up herbicide applications, which is currently limited by water presence (OMNRF 2017b), will influence the success of long-term management, as well as the trait and taxonomic composition of the community. If species are continually wiped out by aggressive herbicide application each season in order to control the speed in which phragmites spreads, this may result in a community that always has traits associated with an early successional community (high specific leaf area, low leaf dry matter content). If the OMNRF is unable to conduct follow-up monitoring due to water levels, this could result in phragmites re-encroachment and a community with high specific leaf area. Furthermore, phragmites rolling, cutting and burning are a management practice employed by the marsh (OMNRF 2017b), but data on the areas that were rolled and burned in 2017 is limited (and is, like herbicide application, dependent on environmental conditions like water levels). In either situation, this may make specific leaf area less reliable measure to track over time. Care will also need to be taken when evaluating community weighted mean results, given that both early successional species and invasive species can have similar traits. It is likely that a combination of trait and taxonomic monitoring may be a better route for large-scale invasive species management projects, or a trait-based study that separates native and invasive trait data.

2.7.2 Traits That Were not Different between Created Ponds

The comparison of trait data between ponds and open marsh were completed to assess whether traits would change with pond age, and whether traits would change between the open water marsh and the created ponds. Community weighted mean for specific leaf area did not show significant differences between ponds (Table 4, Figure 10)**.** In general, variation in specific leaf area has been, and is a commonly discussed characteristic of the trait (Shipley et al. 2016). For example, McCoy-Sulentic et al. (2017) found variation in specific leaf area trait data and ultimately no statistically significant differences between their treatment groups of wetland indicator statuses for plant communities (e.g. obligate, facultative). This was in contrast to their expectations that, since specific leaf area is a key component of plant ecological strategies, there would be significant differences (McCoy-Sulentic et al., 2017). In a 25 years successional study in grasslands, Kahmen & Poschlod (2004) also did not find that specific leaf area changed significantly with succession, though they did not identify whether they used community weighted means or raw values in their analysis.

There are several possible reasons why there were no significant differences in specific leaf area between ponds. While specific leaf area is a commonly selected trait in restoration-oriented studies, there exists a great deal of trait variability that could have either masked or caused no statistically significant differences between ponds. Specifically to wetlands, covariation can occur with leaf traits (Vernescu & Ryser, 2009). Different leaf and plant components can contribute to specific leaf area values in species; for example, mechanical support can be contributed only by a leaf, but in some species mechanical support can also come from the stem, which would result in higher specific leaf area (Hodgson et al., 2011).

Specific leaf area values can also vary within a species. In the successional study by Garnier et al. (2004), they note that changes in community weighted means of their studied traits (which included both specific leaf area and leaf dry matter content) could have been because of interspecific variation (a shift in species with different trait values), intraspecific variation (changes in trait values within species), or a combination of both factors. However, Garner et al.

(2004) notes that similar studies to theirs shows that intraspecific variability was lower than interspecific variability, leading them to conclude that their shifts in community weighted mean values were due to species replacements. The assumption that intraspecific variability is generally less than interspecific variability has been supported by other researchers, but caution must be taken to not overgeneralize (Lavorel e al., 2008; Garnier et al., 2016, p. 108). While traits associated with vegetation are generally similar between dominant species, regenerative traits are less so (Lavorel et al., 2008). Intraspecific variability also varies because of plasticity and genetic variability in populations (Lavorel et al., 2008). The questions of when it is appropriate to disregard intraspecific variation is largely unstudied, and where it has been studied, results are inconclusive (Shipley et al., 2016).

If we do need to consider intraspecific variation, it means that we need to consider trait values for individuals, which is doable, but it means that functional ecology would no longer be as general as it claims to be when it comes to predictability (Shipley et al., 2016). Studies that examine intraspecific variability explicitly are time consuming and complex, as individual studies need to be conducted for a given trait and environmental condition (Garnier et al. 2016, p.108). Furthermore, it is possible for wetland plants to exhibit a high degree of intraspecific trait variability to adapt to high levels of environmental variability, such as water level and water table fluctuations (Moor et al., 2017). For this reason, it is possible that intraspecific variability could have played a role in specific leaf area variation within the Crown Marsh.

In addition to co-variation and intraspecific variability, environmental factors can introduce variability in trait-based studies. Environmental variables can drive trait patterns and changes in communities; traits that respond to environmental changes are well known as ‘response traits’ (Laughlin, 2014). While we are interested in how response traits change with ecosystem change (in the case of restoration, with restoration treatment), there is added variability with wetlands experiencing extremes in these environmental characteristics.

Specific leaf area is not always consistent with highly wet environments, and can increase, show no change or decrease (Moor et al., 2017). Low specific leaf area could be because of a plant trade-off to other structural or physiological traits, or it could be an adaptation to high water levels (Moor et al., 2017). In plants sensitive to flood conditions, increased water saturation can cause a lower specific leaf area, as the drought sensitive plant is going through drought stress due to anoxic soil conditions (McCoy-Sulentic et al., 2017; Moor et al., 2017). Whereas for plant less sensitive to flooding conditions, specific leaf area has the potential to be larger (Moor et al., 2017). Wetlands also change drastically in pH and mechanical disturbance which is not accounted for in traditional plant strategy theories (Moor et al., 2017). This variation in specific leaf area responses to wet environments, combined with the variation in water levels in the Great Lakes and seiche events in Lake Erie (Mortsch et al. 2006) represent two significant causes of environmental variation for trait-based research in coastal wetlands. Water level fluctuations have also limited the ability to develop universal vegetation indices (e.g. IBI) to rank wetland quality in large scale monitoring programs like the Great Lakes Monitoring Program (Albert, 2008).

Finally, given the increase of specific leaf area with herbicide treatment, it is likely that a lack of significance of community weighted means for specific leaf area between ponds is because of restoration treatment variation. Two restorative actions were taken in the Crown Marsh: the creation of dug ponds, and the control of phragmites through herbicide treatments. The treatment of phragmites was conducted through an emergency use permit (targeting large patches of phragmites), and through ground spray on an ad-hoc basis, dependent on water levels and regeneration areas for phragmites (OMNRF, 2017b; Veenhof, 2017c). This resulted in ponds that had all, part, or none of their plots treated for herbicide. This variation in management treatment per pond could have easily been a contributing factor for variation in specific leaf area that resulted in no differences between ponds.

While community weighted means for specific leaf area remained unchanged between ponds, leaf dry matter content was significantly different in Ankney Pond when compared to the other three treatment areas. Leaf dry matter content was highest in Ankney pond overall, which is the oldest pond in this study. The reasoning for this change is less clear; a higher leaf dry matter content with pond age is in contrast to other studies, where leaf dry matter content was found to decrease with site age (Garnier et al., 2004). Once again, it is likely that this difference is because of variation within the site. It is also possible that all ponds, which are all younger than 6 years in age, were not old enough to show differences between in trait values. In a follow-up study to Garnier et al. (2004), Kazakou et al. (2006) also tested species-level (i.e. not weighted means) traits between successional old-field sites. They found that, in general, when comparing their early (3 year) mid (10 year) and late (25 year) successional stages, there was no statistical differences between traits for early and mid-year successional sites. It is possible that the Crown Marsh ponds need more time and additional monitoring before trends become clear, as is common with many ecological indicators (Holl & Aide, 2011), in addition to the challenges of controlling for variation in the marsh.

2.8 Conclusion

In spite of the variable results in this study, specific leaf area is still considered an ideal leaf trait to measure due to its positive, linear relationship with relative growth rate (McCoy-Sulentic et al., 2017; Poorter et al., 2009). Leaf dry matter content has also been argued to be a candidate leaf trait in trait-based studies, as it is more independent of leaf thickness than specific leaf area, so can better identify plants within strategies of resource use which demonstrates global-scale correlations between several leaf traits (Wilson et al., 1999). As one might expect, it is challenging to tease out patterns in specific leaf area and leaf dry matter content with ecosystem changes, either caused by environmental factors or management regimes. Restoration projects often result in ecosystems that vary significantly, in diversity and in function, and we often do not understand the mechanisms behind this variation (Brudvig et al., 2017). Response traits are not only influenced by environmental factors, but also with species interactions (Laughlin, 2014). Teasing out trait-environment and species-trait relationships are challenging. In the case of the Crown Marsh, environmental variables like water level fluctuations, pH, and soil quality and nutrient content were not manipulated as part of the restoration treatment, so they were not measured, but are significant influencers of wetland conditions, and could influence community traits (Lavorel et al., 2007; Moor et al., 2017). Furthermore, seiche events and fluctuating Lake Erie water levels would create wetness conditions that vary significantly over the course of a growing season and from season to season (Mortsch et al., 2006). Overall, wetland studies have demonstrated positive, negative, and no relationship between specific leaf area and wetness (Moor et al., 2017).

Whether the changes observed with specific leaf area and leaf dry matter content in the Crown Marsh are because the OMNRF’s management effort, or because of unmanipulated, but dynamic, environmental variation, cannot be confirmed. There is a need to extrapolate whether a relationship exists between specific leaf area and wetness that can be generalized for application in restoration. This is especially important if we seek to move forward with applying trait based research to wetland restoration, as wetlands remain understudied with regard to trait-based research (Moor et al., 2017). While the application of plant traits to evaluate wetland function is an active field of study, it is further behind than the study of vascular plant traits and terrestrial ecosystem function (Moor et al., 2017). There are several ecological and theory based concepts that need to be strengthened, such as the application of plant strategies to wetland community ecology (Moor et al., 2017). Further research into these concepts is required before we can seek to answer practical wetland restoration questions using traits. From a practical perspective, it will also be important to continue to track phragmites management in the Crown Marsh in order to make meaningful conclusions from monitoring protocols; adaptive management protocols, if not properly tracked, can make interpretation challenging. Until then, specific leaf area could remain only partly useful in restoration projects that contain significant environmental changes and adaptive restoration management.

3. Future Use of Trait Based Monitoring in Restoration Monitoring

I sought to determine whether plant functional traits have a place in restoration monitoring protocols. The selection criteria of an ideal indicator for monitoring project success include that they must be ecologically valuable and robust to ecosystem variability (in that they respond predictably to ecosystem changes), but also must be simple and cost and resource-effective to measure (Dale & Beyeler, 2001). Overall, specific leaf area, and less often leaf dry matter content, remain consistently chosen for trait-based restoration research, because they are part of well established plant life history strategies (e.g. Wright et al., 2004, Weiher et al. 1999, Westoby, 1998) and often respond well to environmental change and manipulation. While they are widely used and perform well in academic studies, there are many theoretical, variability-related and practical hurdles that need to be overcome before leaf traits, and traits in general, can be used reliably to provide conclusive information in practical restoration and resource management projects. The following section details these challenges, with future research opportunities for practical project application.

3.1 Can We Use Traits to Assess Ecological Outcomes for Restoration?

3.1.1 The Challenge of Variation: Ecological and Management Implications

While trait-based applications to restoration projects are a relatively new field of study, several studies have had success with tracking trait changes with restoration and/or management treatment (D'Astous et al., 2013; Engst et al., 2016; Piqueray et al., 2015; Sandel et al., 2011; Zirbel et al., 2017). It was demonstrated in Chapter 2 that specific leaf area increased with herbicide treatment, which is promising for the Crown Marsh. If monitoring continues, it may be possible to monitor specific leaf area changes to demonstrate community-level shifts with herbicide treatment, over time. However, we still do not have sufficient answers for the importance of intraspecific variation in wetland traits, and we don't have predictive relationships between traits and environmental gradients that are measurable (Shipley et al., 2016). Intraspecific variation remains a complex theoretical question that challenges the use of trait-based studies in a general sense for monitoring protocols (Funk et al., 2017; Shipley et al., 2016).

In this study, the inability to account for relationships between traits and environmental conditions was a barrier in eliminating ecological variability. Trait based research is commonly completed along an environmental gradient (Dwyer et al., 2014; May et al., 2013; McCoy-Sulentic et al., 2017; Purcell, 2016). However, the ecological restoration action in the Crown Marsh was relatively simple – it involved herbicide application and soil removal, with select mowing and burning where weather permitted. This means there was no environmental gradient beyond the binary treatments and – at this early stage after treatments - measuring environmental variables wasn't particularly useful. Near-future research should seek to measure baseline values of environmental variables now (in any follow up studies) and then continue as gradients establish over time. For example, water level fluctuations could be incorporated into monitoring protocols, given the variability surrounding trait responses, specifically specific leaf area, and flooding (McCoy-Sulentic et al., 2017; Moor et al., 2017). Water level changes have also been found to be a contributing environmental factor to the spread of phragmites in the Great Lakes, furthering the importance of its consideration (Whyte et al., 2008). In general, trait-based monitoring may be best suited to restoration projects that involve some form of controlled environmental remediation, or in multi-site studies where environmental gradients exist, like hydrology and flooding (e.g. Dawson et al., 2017; McCoy-Sulentic et al., 2017; Purcell, 2016). The continued monitoring of areas of the marsh that have been treated with herbicide, against areas that are not, may allow for trait-based monitoring in the future, though this depends on future herbicide management plans.

Trait based studies that lack an environmental gradient may compare community-aggregated trait values with that of a reference site (D'Astous et al., 2013; Engst et al., 2016; Piqueray et al., 2015; Stefanik & Mitsch, 2012). Anthropogenically caused environmental change has become exacerbated to the point where there are no reference states left in places like the Great Lakes coastal wetlands; as ecosystems become more 'hybrid' or even 'novel', the very existence or utility of reference sites may diminish further (Higgs et al., 2018; Murphy, 2018). If an environmental gradient or a reference site don't exist (yet – or ever), the best path is to monitor community trait changes over time. Plant communities, and community trait patterns, are formed by filtering due to abiotic (e.g. resource availability, disturbance) or biotic (e.g. competition, predation) factors (Lavorel & Garnier, 2002). While some patterns can be relatively simple to quantify, natural gradients like succession are more complex (Lavorel & Garnier, 2002; Suganuma & Durigan, 2015) Succession can be created by a combination of many of the above filters, and combined with complicated trait-filter relationships, this can make causal effects hard to identify (Lavorel and Garnier, 2002; Suganuma & Durigan, 2015). This is likely why studies more commonly utilized monitoring protocols that compared community weighted traits to a reference site or against environmental gradients (but see Piqueray et al., 2015 and Garnier et al., 2004).

While some restoration projects can experience natural successional trajectories (e.g. prescribed burning of grasslands), some do not, at least, when the desired end-point is a historical reference system (Suding et al. 2004). Alternative stable states are a deviation from this end point, and well-established concept in literature; an ecosystem can transition quickly between states, as opposed to slower paced trajectories (Hobbs and Norton 1996). They can be non-linear, behave like a threshold, and are impacted by multiple factors (Hobbs and Norton 1996). Alternative stable states and thresholds have also been applied to restoration; if a degraded ecosystem has not crossed a threshold, it is easier to restore by removing the factor causing degradation than if it has already crossed a threshold (Hobbs and Norton 1996). It is also possible that degraded systems like the Crown Marsh can act as an alternative, stable state for a wetland ecosystem, and can be resilient to restoration efforts, causing unusual successional trajectories (Suding et al. 2004). This resilience can be caused by species effects (e.g. an exotic species invasion), landscape connectivity and seed sources (Suding et al. 2004), all of which are mechanisms by which phragmites invades. In cases like the Crown Marsh where a species does not respond to typical ecosystem dynamics, combined with an arguably degraded seedbank, it is easy for internal, positive feedback loops to form, strengthening overall resilience to restoration (Suding et al., 2004). While much theory surrounding alternative stable states and resilience to restoration is predictive, and more imperative in restoration design and framework development, it is challenging to monitor and assess restoration success where multiple factors could be contributing to thresholds and overall resilience of the Crown Marsh to long term restoration. The removal of phragmites, while the main cause of degradation, may not result in the restoration of the Crown Marsh alone; other management actions may be required (Hobbs & Norton, 1996). The confirmation of whether the Crown Marsh has become a degraded stable state is beyond the capabilities of this study, and beyond most small-scale restoration efforts in general (Suding et al. 2004). However, it will be crucial to investigate other restoration actions in the Crown Marsh beyond treating phragmites and to monitor these actions over time, in order to assess the wetland's long-term successional trajectory.

At the very least, in order to use traits to monitor restoration work over time, restoration treatments (e.g. herbicide treatment) must be applied consistently and tracked over time. Resources, cost and legislation restrictions often prevent this from happening; in the case of the

Crown Marsh, herbicide application was not well documented prior to 2014, and targeted ground application after 2014 could be restricted by the presence of water (OMNRF 2017b). The emergency use permit in 2016 that allowed for well-documented herbicide spray (OMNRF, 2017b) is also not a guaranteed treatment every growing season. In the OMNRF implementation plan for the Long Point region (2017b), it was identified that ground spray 'may' occur in certain areas. Dense phragmites stands were the main target for herbicide application, and sparse patches near pond edges wouldn't have been sprayed to avoid herbicide contact with standing water (Veenhof, 2017c). Post-herbicide treatment continued with an integrative pest management approach, and burning, rolling, and cutting was implemented on treated areas where water levels permitted, but was also not documented in a detailed way that allowed for clear identification for this type of post-monitoring study. If an agency does not track exact restoration treatment areas, post-restoration monitoring is challenging, especially if it is not identified in the planning phases of a project. Unless the teams conducting the ground spray application are willing to identify every polygon treated in their final herbicide spray, which is arguably time consuming, original treatment maps could be unreliable when identifying sprayed areas. While this is not an issue from the perspective of practical management of large-scale projects, it poses a challenge when trying to interpret variation in a restored ecosystem to develop monitoring protocols, and to keep track of treatment areas for follow-up monitoring. While traits are ecologically valuable, there is significant variation that exists in projects in the resource management world preventing their use.

Ecological variability is a limiting factor in using traits to monitor projects receiving significant amounts of resource management. Traits are ideal in controlled experiments where these variable factors can be better controlled. This is especially the case for wetlands, where trait-based research is behind in comparison to terrestrial ecosystems like grasslands and water level fluctuations are a normal variable in coastal wetlands (Moor et al., 2017; Whyte et al., 2008). The potential for the Crown Marsh to be in an alternative stable state and the limits to restoration success caused by positive feedbacks via phragmites invasion must also be considered in future restoration management and subsequent monitoring. Overall, the adaptive management, and often ad-hoc manner in which restoration projects must be implemented, due to time, resources, and legislative constraints, limits the ability to interpret trait-based monitoring.

3.1.2 The Challenge of Complex Functional Diversity Indices: Is Simple Better?

There are many indices proposed to quantify functional diversity and community trait characteristics, and there is not a clear academic consensus on the definition of functional diversity, or which index is best (Li et al., 2017; Mouchet et al., 2010; Ricotta & Moretti, 2011; Schleuter et al., 2010; Villéger et al., 2008). In general, trait-based indices can be divided into two groups; into those that measure mean trait values in a plant community (e.g. community weighted mean), and those that measure the variation of traits in a community (Garnier et al., 2016, p. 99). In the first group of indices, the community weighted mean is one of the simplest, frequently used trait-based indices to characterize a plant community (Funk et al., 2017; Ricotta et al., 2010). It is supported by the mass ratio hypothesis, responds to changes in environmental conditions, is thought to be relatively robust to the exclusion of intraspecific variability, and is robust to differences in species abundance measures, all of which make it an ideal index for comparative studies in community ecology (Grime, 1998; Lavorel et al., 2008; Ricotta & Moretti, 2011; Garnier et al., 2016, p. 99). Some propose that while community weighted means can explain community shifts for the mean value of a trait, Rao's Quadratic Entropy, or Rao's coefficient (Rao 1982) is better to explain trait variability, namely whether species functional traits are converging, diverging or trait dynamics are simply random (Mouchet et al., 2010; Ricotta & Moretti, 2011). Rao's coefficient can also explain whether environmental filters (e.g. low trait dispersion), or limiting similarity, (high trait dispersion), are the reason for community composition (Ricotta & Moretti, 2011). It can also measure both single and multi-trait functional diversity (Ricotta & Moretti, 2011).

An alternative to community weighted mean and Rao’s Quadratic Entropy are more complex indexes that examine functional variability between both individuals and (Leps et al., 2006). Proposed by Mason et al. (2005) and Vileger et al. (2008), they can be broken up into three groups; functional divergence (how far species with high abundances deviate from the centre of functional space), functional evenness (a species distribution within functional niche space) and functional richness (the functional niche space a species occupies) (Li et al., 2017; Mouchet et al., 2010; Villéger et al., 2008). These three indices are independent of one another, and functional evenness and divergence are independent of species richness (Villeger et al., 2008; Mouchet et al., 2010; Garnier et al., 2016, p. 1010). They can be used to interpret a variety of plant community characteristics; for example, low functional evenness means that some niche space is unused, which can suggest that a community is less productive and could be more likely to be invaded by an exotic species (Mason et al., 2005). Furthermore, high functional divergence means that there is more niche differentiation and lower resource competition, which results in better resource use overall and a higher functioning ecosystem (Mason et al., 2005). They are proposed to be superior indices because they have been modified to accommodate single or multi-trait approaches, and measure three types of functional diversity (Villéger et al., 2008).

Researchers often debate the relevance and usefulness of single vs. multi-trait indices, as well as simple vs. complex indices. Both community weighted mean, Rao and the group of functional diversity indices developed by Mason et al. (2005) can be applied to analyze multi-trait datasets. This can be beneficial for several reasons. If we only consider one trait, we may miss relationships between multiple traits that contribute to the functional composition of an ecosystem (Lavorel et al., 2007; Villéger et al., 2008). With regard to community weighted mean, it has been suggested that the addition of more traits can reduce uncertainty when predicting ecosystem characteristics like ecosystem productivity (Lavorel et al., 2007; Ricotta & Moretti, 2011).

This is supported by a more recent literature review by Funk et al. (2017), who suggest that both single and multiple trait indices are important for use in response and effect trait studies on ecosystem dynamics, because we simply do not know enough yet to generalize as to which is better. However, with the addition of multiple traits comes timing and resource constraints (further discussed in section 3.2.2). Multi-trait indices can introduce relationships to species richness, which can alter index results, but are not well understood (Garnier et al., 2016, p. 103). The inclusion of complex indices like those proposed by Mason et al. (2005) and Villeger et al. (2008) add a level of mathematical complexity that is also not well understood from an ecological perspective (Garnier et al., 2016, p. 103). It has been suggested to keep it simple; this includes both new research to develop mathematically simple ways to explain functional diversity, but also to return to simpler, descriptive statistics, such as mean, standard deviation, skewness and kurtosis (Enquist et al., 2015, Garnier et al., 2016, p. 103).

This study sought to achieve a middle ground regarding the simple vs. complex index debate, and to utilize the well-used community weighted mean index, which is arguably more complex than descriptive statistics, but not as complex as those proposed by Mason et al. (2005) and Vileger et al. (2008). While some include community weighted mean as a measure of functional diversity (e.g. Lavorel et al., 2008), most researchers consider community weighted means are the descriptive index of the trait-index world; they are a measure of central tendency, describing the mean value of traits in a community, but do not provide information regarding trait diversity (Ricotta & Moretti, 2011). Community weighted means are perhaps more tractable and comprehensible by most practitioners; more complex indices require more research (and ultimately, time and resources spent) in plant community ecology and associate theory. The use of community weighted mean remains an easy, and therefore recommended index to use in future trait-based research in restoration. Where expertise and resources permit, the suite of indices proposed by Mason et al. (2005) and (Villeger et al. 2008) could be useful in restoration. In order to explore the range of ecological information that can be inferred from trait-based data, it is recommended that functional diversity indices like Rao's coefficient, or the suite proposed by Mason et al. (2005) and Vileger et al. (2008), be applied in future research, especially for restoration sites that focus on invasive species management (see section 3.3).

3.2 Are Traits a Practical Monitoring Measure for Restoration Ecology?

3.2.1 How Much Should We Measure? The 80% Threshold in Community Weighted Mean Sampling.

In this study, the top 1-3 species were collected at each plot during the primary data collection season (2017). This was not sufficient to capture 80% of the plant community, warranting a second field season in order to improve these values. A second field season allowed for more trait data to be collected, raising the overall percent cover captured to 83.4%; when divided into treatment groups, the plots that did not receive herbicide applications were the only treatment that did not meet this 80% threshold.

The measurement of 80% of the plant community is a widely accepted threshold when measuring traits to answer non-evolutionary questions. Both trait manuals used in this study for trait measurements support the 80% threshold. The trait manual by Perez-Harguindeguy et al. (2013) suggests 'greater than' 80% relative abundance is an ideal target; this high number is chosen, according to the original manual of Cornelissen et al. (2003), in order to get an accurate idea of the composition of an ecosystem and extrapolate functional groups from individuals to represent the community. These manuals cite two major studies to support this threshold; Garnier et al. (2004) and Pakeman and Quested (2007). Garnier et al. (2004) measured the top two dominant species in their study for traits and compared this to trait data capturing 80% of the community, and concluded that for some traits (including specific leaf area), measuring the dominant species in a community may be sufficient. Pakeman and Quested (2007) sought to further confirm the results by Garnier et al. (2004), and tested a variety of sampling efforts for several traits. They found that mean trait estimates did decrease in accuracy with decreasing sampling effort, but were small (by 1% when sampling effort dropped from 90% to 80%, and by 5% when effort dropped to 70%). This also varied by site; specific sites ranged from 7-10% deviation from the mean when sampled at the 90% threshold. Pakeman and Quested (2007) concluded that an 80% sampling target is still an ideal threshold when sampling for traits, and traits should not have a large range of values.

There are several tradeoffs with collecting large datasets to hit the 80% threshold for measuring traits in a community. In general, these studies have not been conducted in all ecosystem types, and are often carried out in grassland environments, which function very differently from wetlands (Moor et al. 2017). Generalizations made over what percentage of biomass is ideal to capture may not be applicable across all systems (Pakeman & Quested, 2007). However, time and effort are, unsurprisingly, the biggest drivers when determining how much of a plant community to sample for traits, and thus, a study's accuracy in trait-based measurements.

Species diversity is a driving factor in sampling effort; in diverse communities, it may be necessary to collect trait data for up to 20 species to achieve an 80% sampling target (Lavorel et al., 2008; Pakeman & Quested, 2007). Pakeman and Quested (2007) averaged 8.4 species per site to achieve this target, and Garnier et al. (2004) sampled between 2 and 12 species. Wetlands are commonly made up of a few species that represent the majority of biomass in a community (Keddy et al., 1998), so it may be feasible, in some wetland types, to collect a sufficient number of species within a reasonable amount of time, to meet 80% targets. That being said, in the Crown Marsh, while only a relatively small number of species were collected overall, in order to achieve 80% of species by percent cover, 3-4 species needed to be collected at each plot. When multiplied by 24-26 plots, this represents a significant amount of time not only in field, but in lab, and this is only considering two traits. Furthermore, biomass is the abundance measure suggested in conjunction with this 80% threshold (Cornelissen et al., 2003; Garnier et al., 2004; Pakeman & Quested, 2007; Pérez-Harguindeguy et al., 2013). While biomass is considered one of the best abundance measures in community studies, it is a very time-consuming method to collect, and rapid measures of abundance (e.g percent cover) collection are often selected to cut down on time requirements, especially in large research areas (e.g. percent cover), or in situations where the community weighted mean is being used to assess environmental change (Lavorel et al. 2008).

3.2.2 On the Practical Application of Trait Based Work: Simple and Inexpensive to Measure?

Of the three research questions asked in this study, arguably the most important is whether traits are measurable from a practical perspective. Ecologically, traits may be a superior method to explain plant community characteristics and ecosystem change, and ultimately measure restoration success in restored systems. However, if they are not simple, inexpensive and able to be measured in a time-efficient manner, they will not be utilized by the resource management community to assess actively managed ecosystems, and will remain only a monitoring tool for research purposes.

Both leaf traits were generally easy to collect in the field, but prior to trait collection, species identification posed several challenges. In order to identify many rushes, sedges and grasses, you need to sample species at the time of flowering, and sometimes you need multiple stages of these species' life cycles, in order to get a confirmation on the specific species (Voss & Voss, 2012, pp. 166). For example, in a *Field Manual of Michigan Flora*, the number of stamens separates *Juncus effusus* from three other species (*J. filiformis, J. inflexux and J. balticus)*. Unfortunately, the flowering period was missed for these *Juncus sp.*, making identification challenging. This is especially important if a species flowers early or late in the growing season, when trait collection should be occurring during peak standing biomass, which, in Ontario, is in July and August. While *Juncus effusus* could be identified in this case because of its clump-like growing habit (Voss & Voss, 2012, pp. 167), timing *Juncus* and other wetland species (e.g. *Carex*) flowering and sampling time may not be possible. Some rushes and sedges hybridize with one another (e.g. *Schoenoplectus pungens and Schoenoplectus acutus*, hardstem and softstem bulrsh), making identification increasingly challenging (Voss & Voss, 2012, pp. 148). Some species in the Crown Marsh were also too immature to produce identification parts (e.g. seeds, inflorescence). The challenges associated with proper rush and sedge ID decreases the accuracy of proper species identification, which is necessary for trait collection.

Overall, traits were not expensive to measure in terms of equipment required. Equipment utilized to collect both specific leaf area and leaf dry matter content were standard to any research lab or inexpensive to purchase: plant clippers to obtain samples, plastic bags, paper towel, ice and a cooler for sample transport, and a scanner, drying oven, analytical balance and aluminum foil trays for measuring traits. Except for a drying oven and analytical balance (which are standard pieces of equipment in even small labs), they would be cost effective for a small government or non-government group to collect and calculate. Traits could become expensive to measure when considering the staff time required for collection. In my study, for example, it took a full field season for one field team (2 individuals) to collect data for two traits, that were calculated from the same samples. The addition of more traits will increase the amount of staff required to collect the data, or at the very least, increase the hours required for one field team to collect multi-trait data. For groups that only have one or two summer field technicians, there will be cost trade-offs associated with devoting all their time to trait-based collection.

With staff-related cost requirements, comes time related challenges. If sampling sites are not near a research lab or office, this adds significant time to sampling effort. In the case of the Crown Marsh, Port Rowan was approximately 1.5 hours from the research lab. This not only added extra time for sampling effort, but also the amount of time leaf samples were out of water, which can lead to leaves being compromised before they can be measured (Figure 13). For species that were pulled at the beginning of the sampling day, they could be out of water for up to 5 hours while samples were being collected, plus an additional hour and a half due to transport time, which could affect specific leaf area and leaf dry matter content data. Even with leaf hydration overnight, the variation in time that leaves experienced between plots could have introduced more variability into the dataset. This timing challenge also limited the length of a typical field day (from an ideal 8-9 hours to 3-5 hours) in order to maximize the quality of leaves pulled at the beginning of each sampling day, and increased the number of trips needed to collect leaf trait data in the Crown Marsh. Time was also a driving factor in trait selection; as stated in section 2.2, seed mass was originally selected as key traits to measure in the Crown Marsh. Not all species produced seeds at the same time, and some species (e.g. Typha) were too immature to be producing seeds, making it time consuming and ultimately unfeasible to collect seeds for multiple species in the Crown Marsh.

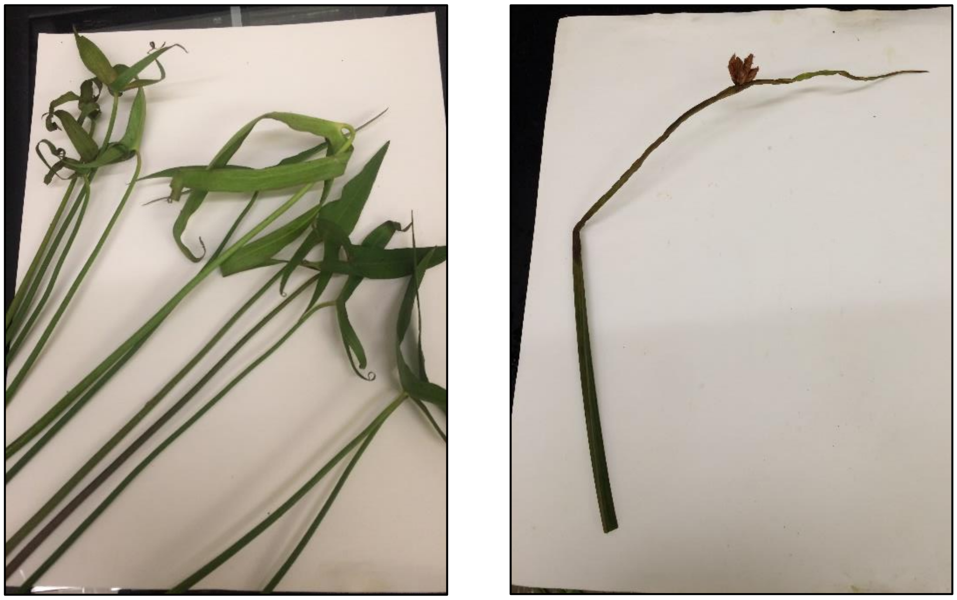


Figure 13: Broadleaf Arrowhead (*Saggitaria latifolia*) and Common Three Square Sedge (*Schoenoplectus pungens*) suspected desiccation from transport and overnight rehydration

Selecting what traits to measure is one of the hardest decisions in trait-based ecology (Funk et al., 2017). This is because it is difficult to anticipate or predict the specific processes driving community or ecosystem-level composition, and the trait(s) that drive those processes (Funk et al., 2017). It has been suggested that designing a study with multiple traits is useful to capture the ecological processes that may be influencing plant community composition (Cornelissen et al., 2003; Engst et al., 2016). For example, leaf traits alone do not explain how plants, especially invasive species, capture and use nutrients; in order to understand how invasive species can expand in poor resource environments, belowground and root traits are important to investigate as well (Drenovsky & James, 2010).

Drenovsky and James (2010) studied both root and leaf traits; they determined, through evaluating root traits (e.g. root length), that the roots of invasive species were more likely to find soil nutrients where they were sparse than native species. The trade-off here is that root traits are difficult to measure (Funk et al., 2017; Lavorel et al. 2007). Furthermore, relationships between root traits and ecosystem functioning (e.g. root traits as an effect trait) are less clear, limiting the ability to make firm conclusions (Funk et al., 2017). Identifying ideal traits to measure is also an arguably costly process in terms of the time required to correctly identify which traits should be measured (e.g. by literature review) (Hallett et al., 2017). The addition of measurable traits also adds more time required to complete data collection, and there is a trade-off between the number of traits one collects and the amount of individual trait data that can be collected. If one chooses to collect a large number of traits, this could result in low plot or site replication for each trait (Cornelissen et al., 2003). In the case of this study, a large dataset for a few traits was chosen over a small dataset of many large traits. By only collecting leaf traits, there is potential to miss other key relationships found in other important traits, such as below-ground and regenerative traits.

While cost requirements were relatively low, processing time was the limiting factor in leaf traits collection. Each plot took thirty minutes to collect leaf samples, and in lab 30-40 minutes were spent processing wet weight, area and dry weights for 10 leaves collected for each species. Based on average sampling effort calculations, if one collects an average of two species per plot, and collects 8 plots in a 4 hour sampling day, one would need a minimum of 8 hours in the lab to process these species. This could increase depending on the number of species sampled in each plot to capture the dominant community, or whether a leaf is difficult to weigh and scan (for example, *Typha spp*. took longer to sample than smaller *Lycopus spp*. leaves). Trial and error could also add time to processing; for example, some wetland species consumed 500mL of water overnight and were found dry the following morning when they were to be processed, warranting recollection. In species like *Typha spp*., samples were visibly losing wet weight while being weighted on the analytical balance during processing, which was a clear indicator of the importance of rehydrating and measuring wet weights as quickly as possible. Finally, space was also a challenge, and while the number of leaves dried per plot varied, leaves took up significant drying oven space. For organizations with only small drying ovens, or those that share ovens with other monitoring protocols (e.g. soil), space will also be a limiting factor in the amount that can be processed in one sitting.

It may not take unrealistic amounts of time to collect species in the field, but it is crucial that a researcher consider the processing time required in lab and not collect too many leaves than can realistically be processed in a day. Trait-based projects in urban areas, especially for soft traits, can utilize community involvement to reduce time and resource constraints on monitoring requirements (e.g. Hallett et al. 2017), but for remote areas like the Crown Marsh, time remains a challenge with regard to trait-based monitoring. It is recommended that for future trait-based studies, a plant inventory be conducted one field season prior to trait collection in order to understand the time and effort requirements associated with sampling effort to increase plant identification accuracy within sampling areas, and to decrease the time spent at each plot when collecting trait data. Assessing a site prior to collecting trait data will also provide insight into the ability to collect a given trait. For example, if a restored community is still too early in succession for the plant community to actively be producing seeds, as was the case for some species in the Crown Marsh, seed mass may not be a viable trait to measure in a community. In spite of these challenges, while time consuming to collect, the investigation of the usefulness of traits representing key life strategies, like plant height and seed mass (Westoby, 1998), in restoration, is also recommended.

3.3 What's Next: Future Trait-Based Approaches to Restoration Projects

There is no black and white answer regarding the superiority of trait-based measurements over taxonomic diversity measures. Diversity is easier to measure but can be less informative of ecosystem function; functional traits can provide more insight into the functional structure of an ecosystem, but as this study has demonstrated, can be time consuming and complicated to assess ecological and management-driven patterns. Measuring diversity is quite simple, generally involving a simple count of species and individuals, but trait-based research that incorporates more complex indices involves multidimensional considerations of trait space (Schuleter et al., 2010), arguably more challenging for a resource manager to analyze and interpret. Progress has been made in the past twenty years with regard to understanding fundamental concepts in trait based research, and applying ecological theory to restoration practice, such as response and effect traits, and functional diversity (Laughlin 2014, Funk et al., 2017). In addition to time and effort requirements, challenges still remain in regards to empirical studies, largely associated with the ability to generalize relationships across a variety of scales, species and ecosystems (Funk et al., 2017). In the case of this study, it was difficult to interpret site-level changes due to the large amount of environmental and management variability within the Crown Marsh. While there have been databases developed in recent years (e.g TRY database, Kattge et al., 2011), that can predict large-scale relationships between plant traits and key life history strategies, these databases are less useful in restoration projects like the Crown Marsh, that seek site-level community information like species interactions and trait-environment variation (Funk et al., 2017). In spite of the identification of research gaps, and subsequent progress, there is still a disconnect between applying functional trait theory to selecting species, as well as the use of relative abundances in restoration design (Laughlin, 2014).

As we continue to fine-tune the field of restoration, there has been a call for restoration to shift to a predictive branch of science (Shipley et al., 2016; Brudvig, 2017). While academic studies will focus on research of trait-ecosystem relationships, there is an opportunity to better utilize traits now in the restoration planning process and incorporated in restoration project design, especially with regard to fighting invasive species and restoring ecosystem services (Funk et al., 2017). One of the first stages of restoration design and subsequent implementation is species selection for the re-establishment of native species (Giannini et al., 2016). During this process, restoration practitioners often manipulate species abundances when manipulating an ecosystem; while this is not necessarily done with traits in mind, it would be easy to implement trait-based theory, like community weighted means, that include species abundance (Laughlin, 2014). It follows that traits could be utilized to select for species that contain traits that would target overall restoration goals. For example, species with high, viable seed production, that disperse easily and have root systems that can function in a variety of soil types are ideal colonizers for degraded sites (Giannini et al., 2016). Alternatively, if one wished for slow nutrient cycling at their restoration site, they could select for species traits that have low specific leaf area and high leaf dry matter content (Laughlin 2014). Though plant selection is less predictable in novel conditions, there is also potential to select plants with traits that can tolerate high disturbances (e.g. high specific leaf area and low leaf dry matter content) (Laughlin 2014, Lavorel et al. 2007).

Overall, there is significant potential in the use of traits in restoration design and implementation. Future restoration research in the Crown Marsh should place primary focus on applying trait-based theory to fight invasive species. The ability of a species to become an invasive varies by ecosystem, so it is difficult to identify a general list of traits that characterize an invader (Funk, 2013). An invader can be excluded by selecting species with a similar functional trait to an invader or by spreading native species traits across their niche space to reduce opportunities for invasive establishment (Funk et al., 2008). The theory of limiting similarity (species with similar functional traits are less likely occupy the same niche), suggests that invading species have different traits than native species, which allows them to establish in available niche space, and eventually become invasive (Funk, 2013; Laughlin, 2014; Laughlin & Laughlin, 2013). By selecting native species with traits similar to an invasive species, supported by the theory of limiting similarity, it may be possible to limit invasive spread (Funk et al. 2008, Laughlin 2014). Specific to specific leaf area, it has been suggested for invasives with high specific leaf area, to select species that have high specific leaf area, but differ in phenology and root depth (Drenovsky & James, 2010). However, in highly disturbed areas with aggressive exotic species, planting species with similar traits may not be effective (Hallett et al., 2017). If a plant community cannot establish prior to species re-invasion, planting species with similar traits may lead to competition between similar native and invasive species may not result in a predominantly native community (Hallett et al., 2017). An alternative to planting species with similar traits, is to select species in restoration planning that have different traits than an invader (niche complementarity) (Laughlin, 2014). This would give native plantings a competitive advantage and allow them to co-exist with invasive species (Hallett et al., 2017; Laughlin, 2014).

It is likely that the application of trait-based ecology in monitoring will need to incorporate measures of diversity and structure with measures of ecosystem function. In a meta-analysis of restoration literature by Brudvig (2011), the application of functional traits to restoration projects were uncommon; only 11% of studies found looked at community functional structure of some kind, with 88% using species richness The incorporation of both structure and function has been supported in literature (Chang et al., 2016; Hallett et al., 2017; Kollmann et al., 2016; Swenson, 2011). For example, Hallett et al. (2017) selected native species cover and diversity as a monitoring measure, but a restoration treatment of planting species with dissimilar traits to fight invasive species in an urban riparian zone. Hallett et al. (2017) suggest a strategy of selecting species with dissimilar traits in areas where it is unlikely that an invasive, exotic species can be completely eliminated, as will likely be the case in a novel ecosystem like the Crown Marsh. Future restoration research in the Crown Marsh could consider experimental designs that test native wetland species with similar and/or dissimilar traits to phragmites and their survivability in a highly disturbed system, but incorporate species diversity and taxonomic measures into post-restoration monitoring. This could be as simple as tracking invasive species cover change over time, or it could incorporate other commonly used taxonomic indices (e.g.

FQI, IBI). The incorporation of these indices will highly depend on the costs associated with conducting such monitoring, especially in conjunction with trait-based monitoring. Large scale protocols have conducted cost-benefit analyses in order to identify the resources required for wetland monitoring (e.g. Albert, 2008). Future research could seek to develop a similar analysis for trait-based monitoring, in order to compare the value and effectiveness of trait-based versus taxonomic monitoring in an industry setting.

3.4 General Conclusions

My study found that the use of traits as a monitoring measure for restoration work is possible, but challenging in situations where ecological and management variability is high, where experimental design cannot compare traits to environmental or site characteristics, and due to time constraints that limited the number of traits that could be collected. These challenges align with current research gaps in trait-based theory, as well as the well-defined challenges in restoration monitoring indicator selection. However, there are several options, moving forward, for application of trait-based ecology in the restoration field. Future research and management opportunities for site-specific trait-based application in restoration, as well as management of the Crown Marsh, are as follows:

1. Continue monitoring specific leaf area and leaf dry matter content in areas that are repeatedly treated for phragmites, in conjunction with diversity measurements, especially with regard to invasive presence, in order to assess long term trends of phragmites management impact on functional traits in the Crown Marsh.

2. In order to better understand and manage successional trajectories for the Crown Marsh, further restoration actions could be taken beyond phragmites treatment. Future research, and subsequent management, should explore the potential for traits to be used as a restoration design measure; to identify and establish species that have dissimilar traits to phragmites, to establish post- herbicide application, to assess their ability to prevent phragmites re-invasion. It will be important to identify the cost (time and monetary) associated with these processes, as they are the driving factors behind monitoring indicator selection.

3. Future trait-specific wetland research should focus on the relationships between wetness regimes and gradients in Great Lakes Coastal Wetlands, and how this affects plant community traits and site-specific variation.

4. Future trait-specific monitoring indicator research should seek to incorporate additional traits, in order to capture a wide range of ecosystem dynamics beyond leaf traits. This could include the exploration of additional indices that can quantify functional variability in a restored system.

5. This study could be repeated to test the time and resource requirements, as well as ecological relevance of root traits and other traits within established life-history theories (e.g. seed mass, plant height) in the Crown Marsh.

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Appendix I: R code for batch processing of leaf areas using the package LeafArea

```
rm(list=ls())
install.packages("LeafArea", dependencies=TRUE)
require(LeafArea)
run.ij(set.directory='~/Documents/imageJ_batch_trial', distance.pixel=236.3065,
known.distance=1, low.size=0.05, check.image=FALSE)
```

Appendix II: Sample R code for treatment group comparisons

```
rm(list=ls())
setwd("/Volumes/Lexar/Data/Field Raw Data")
CWM<-read.csv(file.choose(""), header=T)
attach(CWM)
boxplot(CWM_SLA~Pesticide, main="SLA Changes with Herbicide Treatment", xlab="Pesticide
Treatment", ylab="Community Weighted Mean")
boxplot(CWM_LDMC~Pesticide, main="LDMC Changes with Herbicide Treatment",
xlab="Pesticide Treatment", ylab="Community Weighted Mean")
boxplot(CWM_SLA~Pond, main="SLA Changes between Ponds", xlab="Pond", ylab="Community
Weighted Mean")
boxplot(CWM_LDMC~Pond, main="LDMC Changes between Ponds", xlab="Pond",
ylab="Community Weighted Mean")
library(FSA)
library(car)


#Parametric assumptions

shapiro.test(CWM_SLA)
shapiro.test(CWM_LDMC)
leveneTest(CWM_SLA ~Pesticide)
leveneTest(CWM_LDMC ~Pesticide)
leveneTest(CWM_SLA ~Pond)
leveneTest(CWM_LDMC ~Pond)


#Mann-Whitney U for non-parametric, 2 Groups (Pesticide treatment groups)

wilcox.test(CWM_SLA~Pesticide)
wilcox.test(CWM_LDMC ~Pesticide)

#Kruskal Wallis for non parametric, 3+ Groups (Pond & Open Marsh treatment groups)

kruskal.test(CWM_SLA ~Pond)
kruskal.test(CWM_LDMC ~Pond)

#Dunn post-hoc for Kruskal Wallis

dunnTest(CWM_SLA ~Pond, method='bonferroni')
dunnTest(CWM_LDMC ~Pond, method='bonferroni')
```