THE RELATIVE ROLES OF DISPERSAL AND ESTABLISHMENT FOR SHAPING AQUATIC MACROPHYTE DIVERSITY AND COMMUNITY STRUCTURE AMONG THE INLAND LAKES OF ISLE ROYALE NATIONAL PARK, MICHIGAN, USA.

By

Angela A. De Palma-Dow

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements for
the degree of

Fisheries and Wildlife – Master of Science

2015

ABSTRACT

THE RELATIVE ROLES OF DISPERSAL AND ESTABLISHMENT FOR SHAPING AQUATIC MACROPHYTE DIVERSITY AND COMMUNITY STRUCTURE AMONG THE INLAND LAKES OF ISLE ROYALE NATIONAL PARK, MICHIGAN, USA.

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Aquatic plant (macrophyte) diversity and structure is important for freshwater littoral zone community dynamics, primary production, and ecosystem function. Yet, little research of macrophytes has focused on native macrophyte communities in protected or undisturbed lakes. In our research, we ask what among-lake environmental factors shape native macrophyte richness, diversity, and community structure (i.e. growth form). We identified what relationships exist between these macrophyte metrics and 1) dispersal and introduction potential (i.e. the hydrologic connectivity among water bodies that facilitates new introductions) and 2) establishment and growth potential (i.e. lake and catchment features that determine growth success). To answer our question, we collected physical, chemical, and biological data from 15 connected and isolated inland lakes on Isle Royal National Park (ISRO) during the summers of 2012 - 2013. Results from partial least square regression (PLSR) analyses found that while the drivers of macrophyte communities included measures of 'dispersal and establishment', the most important and recurring predictors of macrophyte richness, diversity, and structure were those corresponding to 'establishment and growth'. Because ISRO is a remote Lake Superior hemi-boreal archipelago that has been a designated wilderness area since 1950, our results provide information about macrophyte reference conditions that can be used when identifying and understanding future responses to pressures such as climate change and invasive species introductions.

I dedicate this work to anyone who has looked under the water and was curious about the wonderful plant life that lives there.

ACKNOWLEDGEMENTS

Thank you to my California and Michigan families for providing me not only the needed support and assistance for me to complete graduate school, but the ability to move away from home to accomplish that dream. The ability to move across the country to further one's education is not an option for everyone, and because of the personal support from my family, I am very lucky that this option was available to me. A special thank you to my partner and love, Jody for being there throughout this endeavor because thanks to you, alongside Wolfy and Rosso, it has been easy to call Michigan my home. I would like to also acknowledge and thank all the members of the Limnology lab at Michigan State University. I moved across the country to live in a new place, with new people, researching a new system unlike anything that I have done before. The graduate school experience was very challenging and your support, friendship, laughter, and advice made the journey not only possible, but enjoyable and pretty darn fun. I will never forget you guys, you are my friends forever; Emi F., Emily N.H., Jeff W., Alex R., Katie D., Paul B., Shikha S. and Caren S. I want to thank my employer, friend, and fellow female in the aquatic sciences, Dr. Jo Latimore, for being a wonderful boss and allowing me the liberties, both in schedule and design, to work with her on the Exotic Aquatic Plant Watch. I have learned much from you Jo and I wish to keep on learning! I am very thankful to the Lyman Briggs College at Michigan State University for providing me with a teaching assistantship throughout the duration of my graduate studies. Not only did this TA position provided me the necessary resources to remain in graduate school, but I learned a great deal about teaching complex scientific concepts, creating inclusive environments, and working as a team with peers, professors and future scientists. Additional acknowledgment to the Institute of Water Resources

at MSU, the College Agriculture and Natural Resources Alumni Association, the Fisheries and Wildlife department and the Graduate school for providing funding for my research. I want to thank my committee members, Dr. Garrett Crow and Dr. Kim Scribner, both very busy and smart men who took the time to provide me with useful and articulate comments, sage advice and words of wisdom about swimming in lakes and counting plants. Thank you to the National Park Service staff of Isle Royale National Park, you made the experience of working far from home within a wilderness area very comfortable and safe, not to mention the tasty ice cream days at Mott.

Of course, I would not have been able to accomplish any of this without my most awesome, wonderful, brilliant and beautiful adviser, Dr. Kendra Spence Cheruvelil. Looking back on the very first time I contacted Kendra about taking me on as a potential student, I think the planets must have been aligned and shooting rays of good fortune directly at me because from the very first email, phone chat, and funding proposal (all while still being a busy undergraduate in California!), Kendra has been magnificent in her desire, support, communication, and guidance for me becoming successful in the aquatic science field, both as an educator and as a researcher. I wish every graduate student could have had such a wonderful adviser like Kendra, whom is not only a remarkable mentor but also a true, wholesome friend. Words cannot articulate how thankful I am for finding you Kendra.

PREFACE

Portions of this thesis were written to be used in a journal publication and in the text “we” is used rather than “I” to reflect the contribution of the coauthor, Kendra Spence Cheruvelil in preparing the manuscript. However, I have done all field work, statistical analyses, literature reviews, and writing of this thesis.

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INTRODUCTION

The roles of macrophytes in lakes

Macrophyte communities are vital to the physical, chemical, and biological structure and function of littoral zones in lake systems. The physical structure and composition of macrophyte communities influence the light penetration and attenuation within the littoral zone (Middelboe & Markager 1997; Squires et al. 2002), and provide heterogeneous habitat for a diverse community of fish, epiphytes, and aquatic macroinvertebrates (Carpenter & Lodge 1986; Sousa et al. 2011). As valuable primary producers, the density and type of macrophyte community can drive seasonal differences in biologically-available oxygen and nutrient levels within the water column (Vis et al. 2006). A diverse and structurally complex macrophyte community also provides habitat and food resources for a variety of aquatic species such as fish, turtles, waterfowl, and invertebrates (e.g. For example, on Isle Royale National Park -- hereafter ISRO), moose obtain necessary sodium from macrophyte grazing, which makes up a large portion of their summertime diet (Aho & Jordan 1979), and beaver dam use and persistence has been positively associated with the presence of specific macrophyte species and biomass (Bergman & Bump *in press*). It is clear that macrophytes serve important roles in lakes, therefore it is important to study the physical, chemical, and biological processes that are shaping and being shaped by these aquatic plant communities.

Compared to other aquatic organisms, macrophytes and their contribution to aquatic communities are generally understudied (Barrett et al. 1993; Hofstra et al. 1995). Although some information exists about how macrophyte communities are best measured and about the relationships that occur between these macrophyte metrics and biotic and abiotic factors, most

past studies have focused on a single functional parameter of a macrophyte, or employed a single diversity index, such as richness, to characterize the entire macrophyte community. In general, there is no one single "best index" or measure that can capture the entire biodiversity and functioning of a given community (Giller et al. 2004; Mikulyuk et al. 2010). Therefore, we described macrophyte communities using a variety of response variables that are known to influence macrophyte communities (i.e., different macrophyte metrics) and to identify the characteristics that shape these aquatic communities.

Although many environmental features can influence the diversity within and among macrophyte communities, most research have focused on lakes that have been under the influence of some form of human-mediated hydromodification, such as dam or weir installation, channelization, cultural eutrophication, and/or biological manipulation by the intentional or non-intentional introduction of non-native and invasive species. Few studies focus on the communities residing in relatively undisturbed ecosystems, although this research focus is necessary in order to best interpret and identify the natural variation in communities and identify measurable standards (Lindo & Gonzalez 2010; Millennium Ecosystem Assessment 2005; Mikulyuk et al. 2010; Chiarucci et al. 2011; O'Hare et al. 2012). In other words, documenting current "natural" composition of macrophyte communities, especially those with limited exposure to human-mediated influences, is essential for determining the magnitudes of future effects that can alter the form and function of aquatic communities.

One such threat to macrophyte communities is biodiversity loss. Loss of diversity is generally of global concern, closely linked with decreased adaptability and loss of valuable ecosystem services (Millennium Ecosystem Assessment 2005; Chambers et al. 2008; Chiarucci et al. 2011). Specifically for macrophytes, biodiversity is important for freshwater lake and

wetland ecosystems because macrophytes provide essential ecosystem services to lakes such as nutrient cycling, sediment stability, improved and maintenance of lake water quality and clarity (Carpenter & Lodge 1986; Scheffer 1998; Sousa et al. 2011). In the face of natural and human-induced stressors, such as changing climate and invasive species introductions, a diverse macrophyte community can play an important role in the resilience and maintenance of aquatic ecosystems (Kennedy et al. 2002; Levine & Antonio 1999; Chambers et al. 2008). In fact, past research has shown that highly diverse and dense communities of native macrophytes can be resistant to potential invaders, particularly in lake systems (Capers et al. 2007; Best et al. 2008; Thum & Lennon 2010). Originally proposed by Elton (1958), the "diversity resistance hypothesis" suggests that invaders have few resources available to facilitate establishment when the native community occupies all available niches. This phenomenon has been substantiated by both observational and experimental research. When observing succession in newly-created reservoirs, it was found that readily-available niches were plentiful where native species density and diversity was low (Havel et al. 2005). Furthermore, when an invasive species was introduced, there was a strong negative relationship between invasive establishment and numbers and types of native species that became established (Naeem et al. 2000; Kennedy et al. 2002). In addition to negatively effecting macrophyte community resilience in the face of changing conditions, decreases in native macrophyte biodiversity may have consequences for macrophyte populations, shallow zone communities, and entire lake ecosystems.

We study pre-invasion or pre-disturbance macrophyte communities in the isolated, freshwater lakes in the wilderness area of Isle Royal National Park (ISRO). ISRO is a hemi-boreal archipelago located in the northwestern portion of Lake Superior, Michigan, U.S.A. Containing 278 inland lakes and ponds (USGS 2008) and measuring over 327 miles of shoreline

(Crane et al. 2006), this wilderness area is a destination location to approximately 15,000 boaters, backpackers, kayakers, fishers and divers every year; it possesses the highest visitor-return rate of any National Park in the U.S. (DuFresne 2002; NPS 2014). Yet, the remoteness and wilderness nature of ISRO provide us with an excellent opportunity to better understand the factors that drive macrophyte diversity in relatively undisturbed lake systems with little influence from altered or highly variable land use. For example, ISRO is home to several rare, Michigan special concern, threatened and endangered macrophyte species, such as alternate-leaved watermilfoil (*Myriophyllum alterniflorum*), aquatic lake cress (*Armoracia lacustris*), Farwell's milfoil (*Myriophyllum farwellii*) (Meeker et al. 2007), and pygmy water lily (*Nymphaea leibergia*) (A. De Palma-Dow, personal observation 2011; Voss & Reznicek, 2012). Invasive macrophyte species, such as Eurasian water milfoil (*Myriophyllum spicatum*), purple loosestrife (*Lythrum salicaria*), and curly leaf pondweed (*Potomogeton crispus*), have not yet become established in the inland lakes on ISRO, although they are routinely found in lakes on the nearby mainland of Michigan, Wisconsin, and Ontario, as well as the shallow zones and shorelines of the surrounding Lake Superior. Our study will add important baseline information about these intact, low-disturbance systems that can be used as a measure of natural heterogeneity and as a comparison in light of future disturbances.

Drivers of diversity and structure in macrophyte communities

Two main factors likely influence the distribution and composition of aquatic plants within lakes: 1) hydrological connectivity that facilitates species introduction and dispersal, i.e. the physical connections between aquatic ecosystems; and 2) the lake and its associated catchment features that promote or inhibit plant growth once a species have been introduced, i.e. the physical, chemical, and biological characteristics of a lake and its catchment (e.g., Leibold et al. 2004; Sousa et al. 2011, Mikulyuk et al. 2010; Akasaka & Takamura 2012; Kissoon et al. 2013). However, since the species diversity of native aquatic plant communities is seldom studied, we know little about which measures of connectivity, the catchment, and the lake tend to drive patterns of native aquatic plant diversity (O'Hare et al. 2012).

One way to identify potential dispersal effects on freshwater macrophyte communities is to characterize the hydrological connectivity among lakes. In this case, hydrological connectivity can be defined as the ability of macrophyte propagules to move across landscapes, from lake to lake through physical hydrological surface connections (i.e. streams, rivers, and wetlands connecting individual lakes). In addition to seed production and dispersal, many macrophytes use fragmentation as a form of asexual reproduction and propagule dispersal that affects their distribution and establishment (Barrett et al. 1993; Laushman 1993). Therefore, the presence of hydrological connections becomes increasingly important – and essential- for species dispersal (Barrett et al. 1993; Dahlgren & Ehrlen 2005; Honnay et al. 2010; Akasaka & Takamura 2012). Identifying the effects of hydrological connectivity on macrophyte communities is therefore important because the combination of aquatic connectivity and the potential for future species introduction, potentially from invasive or aggressive native plants, may lead to drastic changes in biodiversity, as seen in non-aquatic systems (Chisholm et al. 2010; Akasaka & Takamura 2012).

There has been little research on the role of physical hydrological connections in shaping macrophyte diversity (Akasaka & Takamura 2012; O'Hare et al. 2012). However, lakes situated at the end of a connected lake chain, or at the bottom of a drainage basin, contain significantly different chemical composition than lakes closer to the beginning of the chain (Soranno et al. 1999). Genetic studies of bacteria have observed differences between populations in seepage lakes versus drainage lakes that have different sources of water (precipitation, runoff, and supplementing groundwater versus surface water connections to other lakes, wetlands, and streams, respectively) (Yannarell & Triplett 2005). Larson et al. (1995) discovered that while zooplankton and phytoplankton species diversity did not differ between isolated and connected high-alpine lakes, planktonic cell density was greater in isolated lakes than in connected lakes. A study measuring river and lake flood plain inundation duration (i.e., times of extreme river-lake connection due to flood events) found that concurrent with flooding, water and nutrient levels increased, while macrophyte density decreased (Van Geest et al. 2003). Therefore, results of these limnological studies using hydrological connectivity as a predictor of physical lake factors and biological responses provide us with reasons to test its importance for predicting macrophytes.

There are a few studies that include connectivity metrics when determining macrophyte diversity focus on lakes in disturbed or altered landscapes (Dahlgren & Ehrlen 2005). In a system of man-made ponds, macrophyte richness was lower in isolated ponds compared to overall richness in interconnected ponds (Akasaka & Takamura 2012). In Scotland lakes, variation in submersed and emergent macrophyte communities was driven by a combination of environmental variables, such as phosphorous, alkalinity, and connectivity metrics, such as nearest neighbor lake proximity (O'Hare et al. 2012). Sousa et al. (2011) discovered that while

connectivity played a contributing role in determining species composition among connected lakes and rivers, relying on hydrological connections as a predictor variable was difficult because of high seasonal and annual weather variability. In comparison, lake conditions were consistent predictors of lake biota (Sousa et al. 2011). Thus, hydrologic connectivity is likely to play an important-although sometimes conflicting and unpredictable-role in macrophyte species presence and composition in lakes.

Compared to studies of hydrological connectivity, there have been more studies examining the environmental variables that are related to macrophyte distributions, abundances, and presence between lake and landscape (e.g., Hakanson 2005; Capers et al. 2009; Kisson 2013). Physical parameters such as lake size, depth, and water color, and surrounding land use are related to water chemistry and quality, and hence can influence macrophyte metrics (e.g., Squires et al. 2002; Hakanson 2005; Cheruvelil & Sorrano 2008; Capers et al. 2009). Larger lakes, which generally contain more inhabitable littoral zone area, have been shown to contain increased species diversity in some studies (Sondergaard et al. 2005). Specifically, richness has been shown to be positively correlated to littoral area and shoreline development factor (SDF), which is a direct measure of lake area and perimeter (Mikulyuk et al. 2010). The physical properties of lakes can indirectly affect chemical and biological parameters, for example, larger, deep lakes are not particularly likely to be influenced by re-suspension of organic material, which can affect water transparency and the nutrients available for macrophytes (Squires et al. 2002; Hakanson 2005).

Water transparency, often measured as clarity using Secchi disk depth, is one of the strongest correlates of macrophyte biomass (Squires et al. 2002; Hakanson 2002). However, it can be influenced by other lake conditions such as catchment size, lake size, lake depth, water

color, and dissolved organic carbon (DOC) content, as well as the internal processes of lakes such as nutrient loading and sedimentation (Larson et al. 1995; Hakanson 2005). Negatively correlated with water clarity, increasing water color can have negative effects on macrophyte community composition (Nurmberg & Shaw 1999; Estandler et al. 2005). Water color can directly affect the transparency and wavelengths of light penetrating the water column, as well as influence the light available for photosynthesis by submersed species (Chambers & Kalff 1985; Squires et al. 2002; Bromark & Hansson 2005). Water color is influenced by (and is often used as a surrogate measure of) dissolved organic carbon (DOC) components, or humic acid substances, within the water column and can appear as shades of brown or red depending on the material input or material breakdown that occurs within a lake (Cuthbert & Giorgio 1992; Nurmberg & Shaw 1999; Wilson 2010). Limited previous research suggests that some macrophytes in colored, humic lakes can be just as productive both chemically and biologically as clear lakes (Nurmberg & Shaw 1999). Therefore, in addition to Seechi disk depth, water color will be sampled within our study lakes.

Species composition in macrophyte communities can be influenced by nutrients and water chemistry, such as alkalinity (Hellquist 1989; Hakanson & Boulion 2002). For example, relationships have been observed between both alkalinity or pH and the successful establishment of variable-leaf milfoil (*Myriophyllum heterophyllum*) in New Hampshire (Thum & Lennon 2010) and overall macrophyte diversity and dominance in Danish lakes (Søndergaard et al. 2005). Nutrient composition of lake water and sediments has been shown to influence macrophyte richness and biomass, depending on the intake route. Maximum phosphorus and nitrogen uptake by macrophytes in the form of orthophosphate (PO_4^{3-}) and ammonium (NH_4^+), respectively, occurs mostly in the root zone through sediment pore water (Barko 1991). In fact,

the contribution of leaves and shoots in nutrient uptake is relatively low compared to sediment utilization by roots (Wang 2008). However, some species rely heavily on available N and P in the water column, such as coon-tail (*Cerotophylum demersum)*, Canadian water-weed (*Elodea canadensis)*, and water-milfoils (*Myriophyllum* spp.), all of which are abundant in submersed vegetative growth. In order to test for uptake preference on species presence and diversity, our study sampled alkalinity, N, and P from both the sediment and water column.

In our study, we aimed to identify: 1) The macrophyte richness, diversity, and community structure within the inland lakes of Isle Royale National Park and 2) whether these response variables are shaped by dispersal potential measured through hydrological connectivity metrics, or by lake and catchment factors that determine establishment potential (O'Hare et al. 2012). We characterized multiple macrophyte metrics (Richness, Shannon evenness, and Inverse Simpson) that incorporate both richness and abundance of macrophyte species per lake and emphasize different aspects of diversity (e.g., the presence and amount of rare versus common species). In addition, we aimed to identify which of our predictor measures were more important in shaping physical macrophyte community structure (i.e. growth form) within the sampled littoral zone. We addressed these aims by characterizing lake and catchment data, and sampling macrophyte communities and lake physical, chemical, and biological parameters from 15 connected and unconnected inland lakes on ISRO over two field seasons.

MATERIALS AND METHODS

Study Site

Field research was conducted during the summers of 2012 and 2013 on Isle Royale National Park (ISRO). Of the 278 inland lakes and ponds on the island, we chose 15 sample lakes that have minimal seasonal and yearly water fluctuations due to beaver activity, and to best represent the most likely long-term, complete, and diverse populations of aquatic plants for the island (sensu Van Geest et al. 2003). Therefore, we sampled permanent lakes that are relatively large (> 10 ha), relatively deep (> 2 m), were not formed by beaver structures, were relatively accessible within the study timeframe, and were more likely to contain higher numbers of macrophyte species (e.g., Squires et al. 2002; Vestergard & San Jenson 2000). Sample lakes were also chosen that included both connected and unconnected lakes as determined by GIS (ESRI 2011; 6 isolated and 9 connected lakes) (Figure 1). Lakes were considered connected if they were located within the same watershed and shared an aquatic corridor such as a connecting stream, creek, or adjoining wetland that could allow propagule movement from site to site (Larson et al. 1995; Soranno et al. 2009). Final sample lake determinations were made by visual inspection of National Hydrological Dataset (NHD) HUC 9 flow line layer (nhd.usgs.gov).

Lakes were sampled during the warmest months of July and August to maximize the probability of documenting the largest number of species, and to avoid spring-specific snow melt dilution effect on chemical and physical water measurements (Larson et al. 1995; Wetzel 2001). Due to unseasonably long ice duration during the spring and summer of 2013, sampling for that year was postponed until August.

Figure 1: Map of sampled lakes from A) the eastern portion and B) the western part of C) Isle Royale National Park, located within northern Lake Superior and indicated in red. Maps were constructed with 1 meter resolution aerial orthoimagery from the USGS NHD, with other layers courtesy of NPS and David Mechenich (UW-Stevens Point).

Connectivity, Lake, and Catchment Predictor Variables

Assessing Dispersal and Introduction Potential - Connectivity Metrics

Binomial determination was used to describe a lake as either connected or isolated, with connected lakes = 1 and unconnected lakes = 2. To account for the potential of macrophyte fragments moving from one lake to another among the connected lakes, we identified and classified lakes by their placement within their lake chain, with 0 = unconnected, and in a three-chain lake, 1 = headwater, upstream, or first lake in the chain, 2 = middle, and 3 = terminal, downstream, or last lake in chain. To further identify potential for new plant material to enter or exit a lake, we identified the number of inflowing and outflowing hydrological connections present for each lake. These connections were identified by NPS topography maps and verified on-site during sampling.

Assessing Establishment and Growth Potential - Lake and Catchment Metrics

Lake and catchment morphometric data used in this study included lake surface area and perimeter, catchment area, and lake maximum depth (Meeker et al. 2011). Shoreline development ratio (SDF) was calculated using lake perimeter and lake surface area by the method of Kalff (2002), which represents the irregularity of a lake, with a 1 value representing a lake that is a perfect circle. Prior to sampling, each lake was divided into quadrants to include every cardinal direction (N, S, E, W or NE, NW, SE, SW, depending on lake orientation; Figure 2). We took samples for chemical parameters and macrophytes from one site per quadrant per lake, and chose those sites randomly in the field to account for any daily wind or fetch effects.

Water clarity using Secchi disk depth (meters) was measured in four randomly selected pelagic sites, one in each quadrant. Water and sediment chemistry data were collected from a total of eight locations within each lake, four in the pelagic zone and four in the littoral zone of

each quadrant. Pelagic water samples were extracted from a 1-m integrated tube sampler operated by a two-person team in an inflatable kayak. Littoral zone water samples were collected using a grab sample taken from 10 cm below the water surface for alkalinity and water color analysis. These analyses were conducted immediately on site using a LaMott Alkalinity Titration Kit WAT-MO-DR and a HACH color test kit CO-1 (item 2234-00), respectively. Sediment alkalinity water samples were extracted from the same location by a 1-m-tall PVC pore-water syringe-powered extractor that we inserted to a soil depth of approximately 10 cm (Winger & Lasier 1991). Water for sediment N and P chemical analysis was stored in portable coolers with multiple instant cold packs until temporarily stored in freezers at ISRO NPS headquarters, then transported and thawed for analysis in the limnology laboratory at MSU. Nitrogen from ammonia (NH_4^+) was analyzed from filtered water samples following Solorzano (1969) protocol. Total Phosphorous (TP) concentrations were determined from unfiltered water samples following the protocol outlined in Menzel and Corwin (1965).

Macrophyte Sampling and Macrophyte Response Variables

Macrophytes were sampled by snorkel survey as described and recommended by Capers et al. (2009) in each of the 15 inland study lakes. To limit effects of uneven sampling effort and subsample bias, species richness and relative abundance were measured from four 50 m perpendicular transects, one within each pre-determined quadrant in each lake (Figure 2). The diversity metrics calculated were richness, Shannon Evenness, Inverse Simpson Index, and growth form. These are described in detail below.

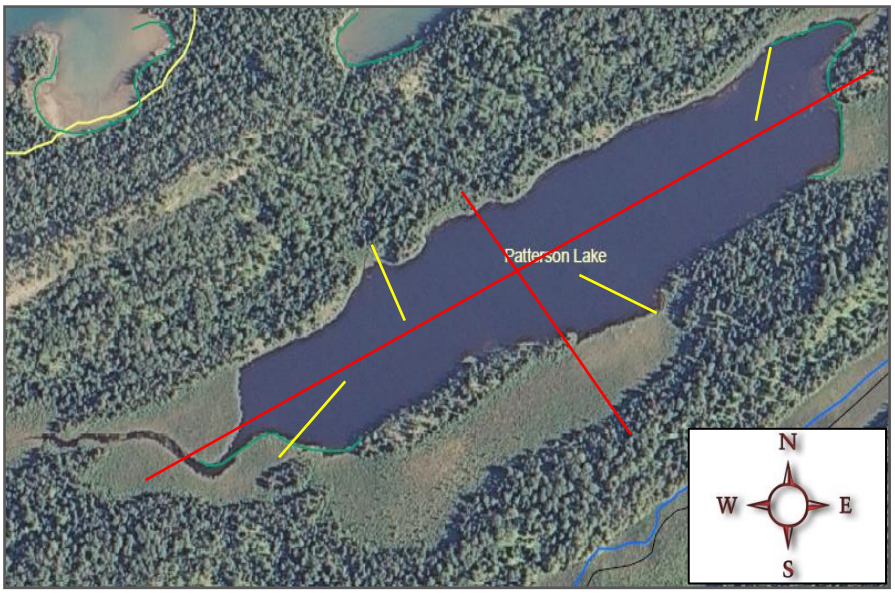

Figure 2: Patterson Lake located on north east end of ISRO being shown as an example of cardinal lake quadrants and semi-stratified transect placement. Red lines delineate the quadrant designations while yellow lines designate approximate site of sampling transects.

Species richness

Species richness was determined by recording the presence of each plant species observed within the lake, regardless of where it was found (Egertson et al.2004). If a species was seen at any point during lake sampling, either as a fragment, rooted or floating specimen, it was recorded as "present" in that lake and a voucher specimen was collected and pressed for final identification according to Crow and Hellquist (2000). Nomenclature followed that of Field Manual of Michigan Flora (Voss and Rexnicek 2012). Voucher specimens were submitted to the Michigan State University Herbarium as part of the NPS Interior Collection Management System.

Macrophyte abundance

Relative abundance of each species within each lake was measured using a combined transect and quadrat semi-stratified sampling regime similar to the methods described by Titus

(1993) and Capers et al. (2007, 2009). Within each lake quadrant, one 50-m field-based "informed" transect was placed perpendicular to shore in an area that best represented that quadrant's most common littoral zone community (Figure 2). This location was determined by swimming along the littoral zone in the designated quadrant and choosing an area that best-represented that quadrant's shoreline and littoral zone vegetation community. The 50 m transect was marked at pre-determined intervals of 0, 5, 10, 15, 20, 30, 40, and 50 m, with the 50 m marker weighted with a rope to the depth of 4.57 m (Capers et al. 2007, 2009), which is the maximum dive depth of the research snorkeler and close to the 5.0 m depth of maximum depth of colonization (MDC) of macrophytes in Wisconsin lakes (Mikulyuk et al. 2010).

Macrophyte species percent occurrence (i.e. hereafter referred to as "abundance") was estimated from a 50 * 50 cm^2 quadrat randomly tossed near each of the eight, marked intervals along the transect line. Within each quadrat at the water surface and the sediment level, abundance of each species was estimated as a single value (i.e. 25%, 50%). In the case that a single value could not be estimated, a range of values similar to Braun-Blanquet subjective cover class was assigned (i.e., < 5%, 5 - 25%, 25 - 50%, 50 - 75%, 75 - 100%; Braun-Blanquet 1964; Llamavirta & Toivonen 1986; Titus 1993; Capers et al. 2007; Engloner 2012). Abundance was estimated for any plant that was present in, on, intersecting, floating, sitting, rooted in, or resting on or in the quadrat at time of sampling. After surface abundance was estimated, the snorkeler pushed the quadrat down through the water column to the sediment and estimated the percent abundance of each species present within the quadrat rooting, sitting, or floating near the sediment. Range abundance values were converted to single mean values using the Engloner (2012) mean conversion so that abundances could be averaged per transect and aggregated into a single value per species for each lake for comparison across lakes.

Classifying community structure by growth type

We use the term 'community structure' to describe the physical structure of the macrophyte community within the sampled littoral zone. To quantify macrophyte community structure, we categorized each species found in each lake by growth type (Figure 3). Species were categorized into one of the following four growth types: emergent, submersed, floating-leaved, and free-floating (sensu Borman et al. 1997, after Arber 1920 & Sculthorpe 1967). The number of species of each growth type was summed and divided by the total number of species found in the lake to obtain the percentage of each growth type per lake. These values describe the community structure of each macrophyte community per lake.

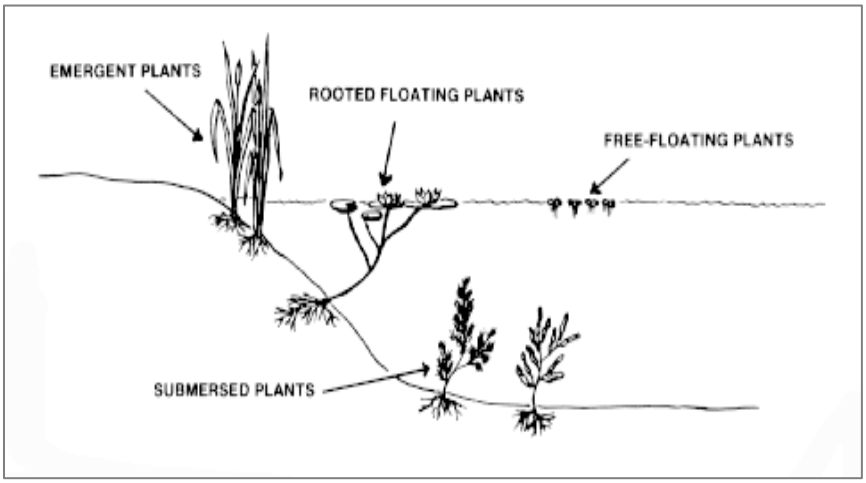

Figure 3: Categories of plant growth types. Emergent species contain a portion of stem or leaf structure that emerges from the surface of the water while maintaining a submersed root zone in the sediment. Submersed species are macrophytes with roots in the sediment, that are entirely under the surface of the water (although there can be exceptions of some floating leaves and reproductive structures that break the water surface or rise out of the water). Floating-leaved species are rooted macrophytes that have mature leaves that sit on or are slightly elevated from the surface of the water. Free-floating species are those macrophytes that do not have roots anchored in the sediment and float at the surface of the water. Photo credit: http://www.uky.edu/Ag/PAT/cat5/cat5.htm

Quantitative methods

Calculating macrophyte relative abundance

We used a version of Braun-Blanquet (1964) cover class designation and an Engloner (2012) conversion method to quantify the amount of each species present within the eight surface and submersed quadrats sampled along the four transects per lake. Using this method of aggregation and averaging of abundance from each quadrat, we determined the relative abundance of each species per lake (sensu Egertson et al. 2004). Most macrophyte studies determining abundance at the species level have employed some form of conversion based on this or similar cover class designations (Egertson et al. 2004; Capers et al. 2009; Mikulyuk et al. 2010; Engloner 2012). After each conversion value was calculated for a given species in each quadrat, the mean abundance along each transect was calculated, followed by a lake-wide mean abundance value for each species. Raw abundances were converted to relative abundance index values (whereby the total value for each lake = 1) using vegan package in R (Oksanen et al. 2013). In this way, a single value representing the mean relative abundance (i.e. percent occurrence) of each species for each lake can be used when calculating different metrics of diversity and comparing these communities across lakes.

Calculating macrophyte diversity metrics

We calculated several diversity metrics for each lake using the mean converted species abundances described above. Multiple diversity metrics were calculated for this study because there is not one metric that captures all of the important information relevant for understanding the contribution of both rare and common species to the overall macrophyte community (Chiarrucci et al. 2011; Englonger 2013). To characterize rare species within a lake, the Shannon evenness (H_E; Eq. 1.1) metric was calculated. This index describes the uncertainty of

selecting a specific individual species picked at random from the dataset (Tuomisto 2010), where the maximum value is 1 and the minimum is 0, meaning that all species are even (and the lake contains maximum diversity) and the species are completely non-even (and the lake contains minimum diversity), respectively. This evenness-specific metric simultaneously increases the contribution of rare species (i.e. low abundance) and reduces the contribution of common (i.e. high abundance) species. In contrast, the Inverse Simpson (D_{invS}, Equation 1.2), is a proportional abundance metric that emphasizes common species and can be interpreted as the reciprocal of the probability that two individuals selected from the dataset will be of the same species with the minimum value, lowest observed diversity, being 1 and the maximum value being the maximum number of individual species at one site, and the most diverse. Rare species (i.e. those with low abundance) have relatively little influence on this overall metric value, resulting in common species contributing more to the Simpson index. The Inverse Simpson diversity metric was chosen to ensure that results could be accurately interpreted because as the reciprocal index value increases, so does diversity (Zhou et al. 2002).

Shannon Evenness **Eq. 1.1**

$$H_E = \frac{-\sum[pi \ln(pi)]}{\ln S}$$

Inverse Simpson **Eq. 1.2**

$$D_{invS} = \frac{1}{\sum p_i^2}$$

Where S = number of species in the sample or Richness; p_i = the proportion (relative abundance) of individuals in the *i*th species. The series of equations used to calculate the species diversity metrics, as described in Chiarucci et al. (2011), were calculated using vegan package (R and Oksanen et al. 2013).

Data Analysis

We used partial least square regression (PLSR) to understand how macrophyte species richness (n = 15 lakes) and community structure percentages were related to connectivity, lake, and catchment variables using the PLS package in R (R Core Team and Mevik et al. 2013). PLSR was the appropriate analytical technique to use because the number of observed response variables was lower than the number of predictor variables in the model, many of the desired variables were highly correlated (identified at absolute value $r > 0.6$ using the CARET package in R), and yet an ecologically meaningful R^2 value was obtainable (Carrascal et al. 2009). PLSR involves a two-step process whereby correlated predictor variables are first consolidated into descending-value contributing 'components'. Then, component scores are used in multiple regressions against responses (e.g., Richness, % emergent, or % floating-leaved). Essentially, this method allows multiple, highly correlated predictor variables to be identified as explaining the variation observed in each response variable. Once all PLSR outputs were summarized, with contributing predictor variables for each model being considered most important when their squared loading weight values equaled ≥ 0.14, the recurring explanatory predictors were identified and the relative importance of introduction/dispersal (i.e. connectivity) and establishment (i.e. lake and catchment features) were determined.

RESULTS

Characterizing study lakes

Physical and chemical variables

Sampled lakes exhibited a range of physical attributes with the largest study lake being 427.8 ha surface area and 16.8 meters deep (Lake Desor 2013) and the smallest study lake being 10.1 ha and 4.0 meters deep (Patterson 2013; Table 1). The samples lakes generally, are relatively small (range = 10.1 – 427.8 ha), relatively shallow (maximum depth range = 3.0 – 16.8 m), and are relatively productive (TP water column range = 6.9 - 86.2 ug/L, and TP sediment range = 30.2 – 199.8 ug/L; Table 2). These lakes are relatively low in clarity (Secchi disk depth value range = 0.9 – 2.7 m) and are highly colored (Apparent color range = 12.5 – 325.0 Co/Pt units).

Table 1: Study lake descriptive statistics. Coordinates are provided for identification of the general lake location and not specific to any one particular sample location; connectivity types are: 1 = connected, 0 = isolated / unconnected lake; and position in lake chain values are: 0 = non-connected lake, 1 = top/headwater lake, 2 = bottom/terminal lake in two-lake chain and middle lake in a 3-lake chain, 3 = bottom/terminal lake. Lakes are in alphabetical order.

Site information				Morphometric metrics					Connectivity metrics			
Lake Name	**Year sampled**	**Latitude**	**Longitude**	**Lake Area (ha)**	**Lake Perimeter (m)**	**SDF**	**Watershed Area(m^2)**	**Max depth (m)**	**No. inflow**	**No. outflow**	**Connectivity type**	**Location in lake chain**
Ahmik	2012	48.149453	-88.539367	10.3	715.9	62.9	455.1	3.0	0	0	1	1
Angleworm	2012	48.084702	-88.64783	50.4	2271.2	90.3	2265.1	9.1	1	1	1	1
Beaver	2012	48.081261	-88.754432	20.1	936.6	58.9	447.7	5.2	1	1	1	2
Benson	2012	48.087282	-88.632166	24.1	961.4	55.3	276.2	4.3	0	1	0	0
Chickenbone	2012	48.065433	-88.72452	92.6	2696.5	79.0	1698.5	6.4	5	1	1	2
Desor	2013	47.975099	-88.987396	427.8	3925.1	53.5	2428.1	16.8	1	1	0	0
Feltdman	2013	47.855675	-89.171246	185.8	2006.9	41.5	1342.6	3.0	1	1	0	0
LaSage	2012	48.05766	-88.710529	45.0	1741.5	73.2	2265.1	9.1	1	1	1	2
Livermore	2012	48.064716	-88.708427	30.1	1166.8	60.0	1698.5	5.8	2	1	1	1
Mason	2013	48.037721	-88.635942	22.8	1201.8	71.0	612.5	7.9	1	1	0	0
McDonald	2012	48.08883	-88.73203	14.8	730.0	53.5	447.7	4.3	0	1	1	1
Ojibway	2012	48.101614	-88.609721	15.7	933.1	66.4	815.3	4.6	0	0	0	0
Otter	2012	48.077419	-88.752071	20.2	883.1	55.4	447.7	4.3	0	1	0	0
Patterson	2012	48.142637	-88.551013	10.1	606.9	53.8	455.1	4.0	0	0	1	2
Richie	2013	48.043632	-88.696067	216.2	3852.4	73.9	2265.1	11.9	4	1	1	3

Table 2: Study lake descriptive statistics of chemical characteristics (n = 15 inland lakes sampled during 2012 and 2013 on Isle Royale). Average values are calculated from four replicate samples taken from each lake.

Parameter	Units	Mean	Standard Deviation	Min	Max	Range
Water Color	Co/Pt	142.9	93	12.5	325.0	312.5
Secchi Depth	m	1.8	1	0.9	2.7	1.9
Alkalinity, water column	CaCo3	66.4	16	31.0	91.5	60.5
NH4, water column**	ug/L	143.1	349	11.3	1343.1	1331.8
TP, water column	ug/L	34.6	25	6.9	86.2	79.3
Alkalinity, sediment	CaCo3	88.5	28	42.5	143.5	101.0
NH4, sediment	ug/L	293.1	418	5.4	1515.8	1510.5
TP, sediment*	ug/L	90.8	55	38.2	199.8	161.6

*Sediment TP for Lake Ojibway based on (n = 2) and McDonald (n = 3).

** One lake (McDonald) had unusually high nitrogen values (n = 4). These values are typical of a highly eutrophic lake and are quite uncommon from a lake on ISRO. This lake was sampled once during 2012 and we analyzed nitrogen by spectrophotometer analysis on two separate occasions to account for processing errors. This result is unexplained and NPS does not regularly sample this lake in their annual monitoring and cannot be confirmed (Elias & Damstra 2011). However, the variable did not interact or appear to influence statistical output of models despite its unusually high values.

Macrophyte communities

We found a total of 56 species across the 15 sample lakes on ISRO, with McDonald Lake being most species rich and Lake Ojibway being least rich (richness = 24 and 9, respectively; Figure 4). *Carex* spp. was found in 13 of the 15 sample lakes, making it the most frequently occurring genera observed across lakes, with a relevant abundance of 0.09. *Potomogeton zosterformis* (flat-stem pondweed) was the most abundant species with a relative mean abundance of 0.06. Seventeen identified species were considered "rare plants", being found in just one sample lakes (Complete species list in APPENDIX 1, Table A1). The distribution of submersed, emergent, and floating-leaved, plants was variable across lakes (Figure 5). However, submersed species comprised the majority of the macrophyte structure, with emergent species secondly adding to the majority of community structure, and floating-leaved species contributed the least to community structure (Figure 6).

Shannon evenness diversity index, which best characterizes rare species, was relatively homogeneous across lakes with a mean of 0.8 (SD $\pm$ 0.1) and a range from 0.4 to 0.9. Inverse Simpson diversity index, which best characterizes common species, was more heterogeneous across lakes with a mean value of 5.9 (SD $\pm$ 2.3) and a range from 1.7 to 10.2. While both metrics found Chickenbone to be the most diverse lake and Feldtman to be the least diverse lake, there were differences between the two metrics of diversity when the lakes were ordered from least to most diverse (Table 3).

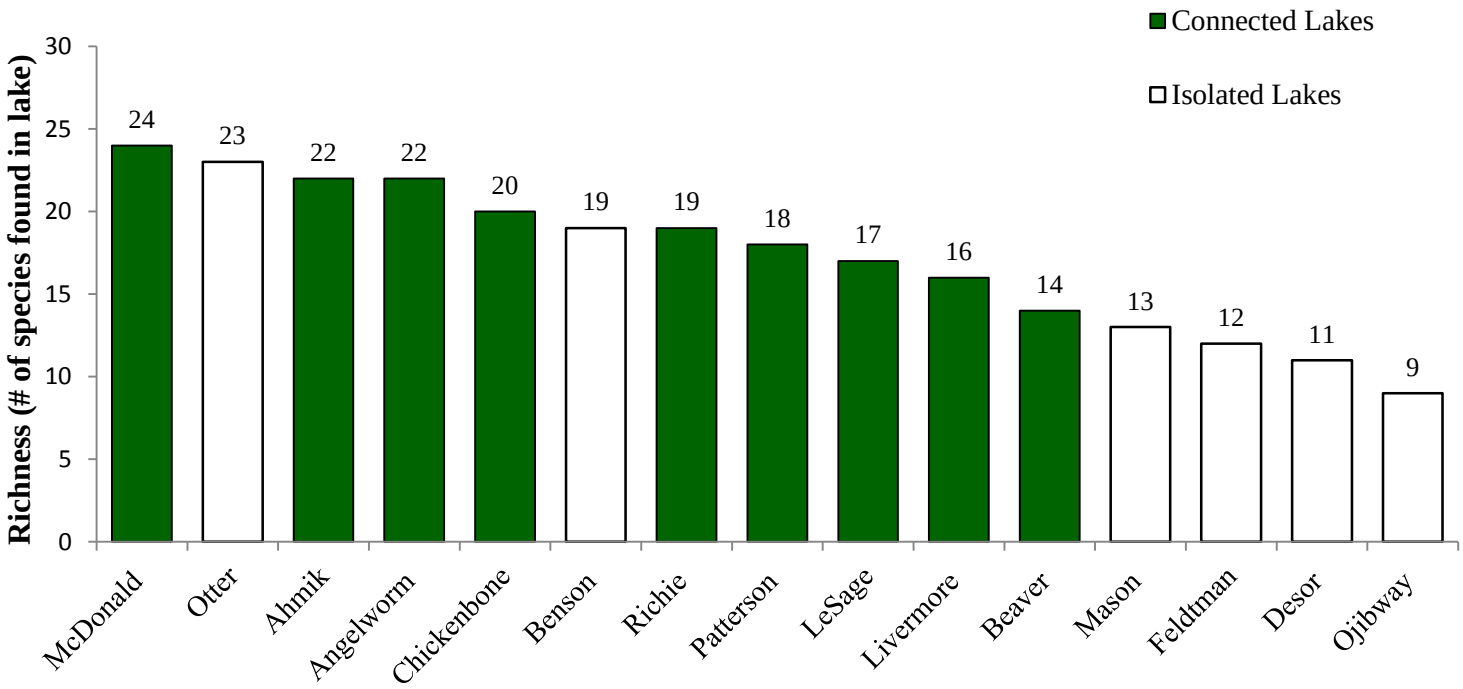

Figure 4: Macrophyte richness per lake (number of species found in each of n = 15 lakes on ISRO 2012-2013), in order of decreasing richness from left to right, with data labels reflecting richness values for each lake. Striped columns represent non-connected or isolated lakes and shaded columns represent the connected lakes.

Table 3: Diversity response metrics, Shannon Evenness and Inverse Simpson index. Response values are listed in ascending order from least diverse near top of table to most diverse towards the bottom. Mean, minimum and maximum values and standard deviation (SD) of values from all lakes (n = 15) is located at the bottom of the table.

Lake Name	Shannon Evenness
Feldtman	0.42
Benson	0.60
Ojibway	0.61
LeSage	0.73
Beaver	0.74
Livermore	0.75
Angelworm	0.78
Otter	0.79
Mason	0.81
Richie	0.82
Desor	0.83
Ahmik	0.83
Patterson	0.84
McDonald	0.84
Chickenbone	0.87
MEAN	0.8
SD	0.1
Min	0.4
Max	0.9

Least diverse

Most diverse

Lake name	Inverse Simpson
Feldtman	1.69
Benson	3.13
Ojibway	3.15
LeSage	3.85
Desor	4.49
Livermore	4.63
Beaver	5.31
Angelworm	5.97
Patterson	7.21
Richie	7.24
McDonald	7.88
Mason	8.05
Ahmik	8.22
Otter	8.26
Chickenbone	10.16
MEAN	5.9
SD	2.3
Min	1.7
Max	10.2

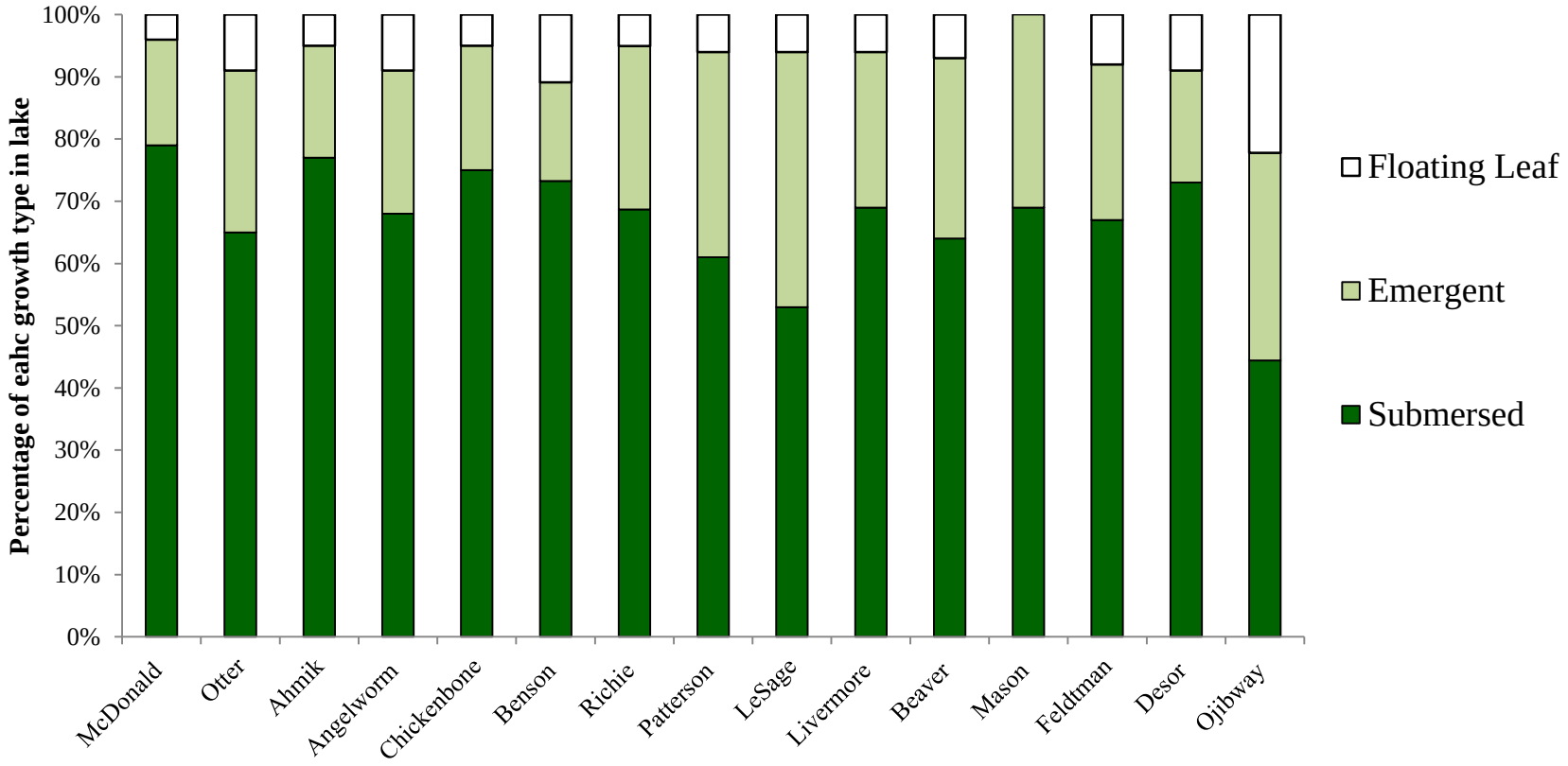

Figure 5: Macrophyte community structure of sampled lakes on ISRO, in order of highest richness lake to lowest richness lake left to right. Distribution of percentage of species in each lake by growth type. For more details on these species growth form distinctions please refer to the Figure 3.

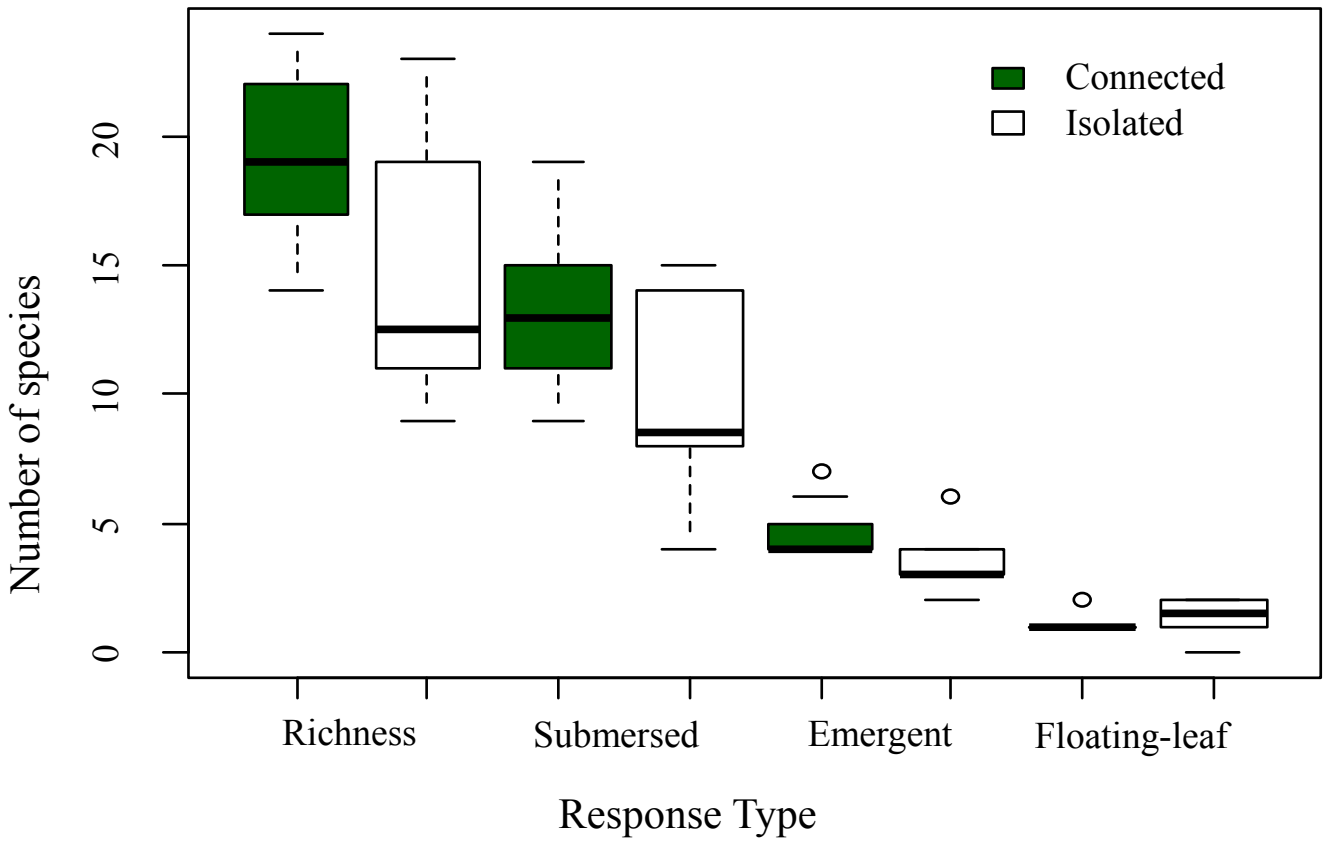

Figure 6: Boxplots showing the number of species for several responses. Based on a series of two-tail T-tests, in each response type, richness (t = 2.36, p = 0.10), submersed (t = 2.23,p = 0.11), emergent (t = 2.26, p = 0.09) and floating-leaf (t = 2.45, p = 0.55), there was not a significant difference ($\alpha = 0.05$) in the number of species between connected (green) and non-connected lakes (white).

Assessing the Relative Roles of Dispersal/Introduction Potential and Establishment/Growth Potential for Structuring Macrophyte Communities

When choosing which predictor variables to include in PLSR models of macrophyte diversity metrics, we considered both multi-colinearity and *a priori* understanding of what drives macrophyte communities. Because connectivity type was correlated with chain location ($r = 0.85$), and was a binary variable that would have interrupted proper PLSR analysis, we excluded this variable from our PLSR models and only included three dispersal variables in models (Appendix 1, Table A4): number of inflows, number of outflows, and position in lake chain. We also pared down the number of lake morphometric variables to include in any one model since they were highly correlated. For example, because perimeter was highly correlated with lake area ($r = 0.86$), watershed size ($r = 0.84$), and maximum depth ($r = 0.83$), this metric was dropped from all models. Finally, because sediment alkalinity is not a regularly sampled limnological parameter, and this variable was correlated with water column alkalinity at $r = 0.85$, we only included water column alkalinity in models. The final list of variables included in modeling efforts is listed in Appendix 1, Table A4 and Table A5.

The PLSR model resulted in first components explaining 59%, 60%, and 56% of the cumulative variation in species richness, Shannon evenness, and Inverse Simpson, respectively ($p < 0.001$). For community structure, the first component significantly ($p < 0.05$) explained 50% of the cumulative variation for submersed species, 40% for emergent species, and 61% floating leaf species.

Connectivity type as a factor of macrophyte community metrics

The relationships between lake connectivity type (i.e. connected vs. isolated) and species richness and growth type were not generally significant (Figure 6). However, the

range for richness was larger, and lower richness values were more common for isolated lakes than for connected lakes. This trend is similar for the number of species within each growth type category (Figure 6), with the highest amount of species being submersed and floating-leaved species being the present in the least amount across lakes. There was also not a significant difference in diversity between connected and isolated lakes using either of the two diversity metrics (Shannon evenness $t = 2.45$, $p = 0.12$; Inverse Simpson $t = 2.31$, p-value 0.18). Overall, lake connectivity type was not a significant factor for richness, diversity, or community structure; however other metrics of connectivity might be important in explaining variation in the responses.

Connectivity Metrics: Introduction and dispersal potential

In the 15 ISRO study lakes, dispersal metrics contributed less than establishment and growth variables in explaining variation in macrophyte communities. However, at least one dispersal, or connectivity, metric was included on the first or second component, with a total of 39-82% variation across the macrophyte response variables being explained by dispersal (Table 4 & 5). Interestingly, the most important dispersal metric included in each model varied by response variable.

There were no contributing introduction and dispersal predictor variables that explained variation in species richness. Likewise, for both Shannon evenness and Inverse Simpson, connectivity metrics were not important contributing predictors for component one (loading weights <0.14). Although some connectivity metrics, outflow for Shannon evenness (-0.16) and lake chain location (-0.34) for Inverse Simpson, did contribute to the second components, these components were not significant ($p > 0.05$) for explaining variation in these macrophyte diversity metrics.

For community structure, only one of the three growth forms was explained by dispersal variables (Table 5). For floating-leaf species, outflow (-0.14) and lake chain location (-0.15) negatively contributed to the significant first component ($p < 0.001$). Although lake chain location (0.19) contributed to the second component for emergent species, that component was not significant ($p = 0.127$). Overall, dispersal metrics were included at least once within the first two components for all macrophyte response metrics. However, dispersal metrics only *significantly* contributed to explaining variation in the metrics of Shannon evenness diversity and community structure as measured by floating-leaf species.

Lake and Catchment Metrics: Establishment and growth potential

For the 15 ISRO study lakes, establishment and growth measured by lake and catchment variables explained more variation in macrophyte communities than did introduction and dispersal metrics. Multiple lake and catchment variables contributed to the first and second components for all macrophyte response variables, but the combination of predictors for each model differed by macrophyte metric.

For richness, the first component included sediment TP (-0.27), alkalinity (0.17), and lake area (-0.13). For Shannon evenness, the variables that contributed the most were alkalinity (0.31) and sediment TP (-0.22; $p < 0.01$; Table 4). For Inverse Simpson, water column alkalinity (0.31) and SDF (0.14) were the only significantly contributing variables ($p<0.01$; Table 4). For richness and both diversity metrics, alkalinity and sediment TP were the most important contributing variables to overall variation.

For community structure response variables, all three growth forms were explained by establishment and growth metrics (Table 5), but with different combinations

of most important contributing predictors. For submersed species, variation was explained by the first ($p<0.01$) and second components (marginally significant at $p<0.08$). The first component included solely alkalinity (0.51), whereas the second component included water column TP (-0.19), watershed area (-0.15), water color (0.15), and maximum depth (-0.16). For emergent species, component one included only alkalinity (-0.34, $p<0.01$). Two components were significant in explaining variation in floating-leaf species. The first component ($p = 0.001$) included alkalinity (-0.34) and water color (-0.14), whereas the second component (marginally significant at $p = 0.08$) included water color (-0.35). Therefore, a wide range of lake and catchment variables explained the variation observed in macrophyte metrics that indicate richness, diversity, and community structure. Generally, alkalinity, sediment TP, and water color most consistently contributed to the variation observed in these lakes (Table 4 & 5).

Table 4: PLSR results (contribution effect and direction) for three diversity response variables: richness, Shannon Evenness, and Inverse Simpson. Variation explained (R^2) and significance (p<-0.05 unless indicated with an asterisk that means p-value is marginally significant at 0.08) reported are for the two first components combined, with most contributing (squared loading weight >14%) predictor variables in bold.

	Richness		**Shannon Evenness**		**Simpson Inverse**	
	Comp1	**Comp2**	**Comp1**	**Comp2**	**Comp1**	**Comp2**
R^2	58.74	68.50	59.50	73.89	56.46	69.90
P-value	0.001	0.25	0.00	0.16	0.00	0.18
Connectivity variables						
# inflow	0.00	-0.01	0.05	-0.06	0.12	0.00
# outflow	0.00	0.04	0.00	**-0.16**	0.00	0.00
lake chain location	0.09	-0.09	**0.14**	-0.02	0.13	**-0.34**
Lake and Landscape variables						
SDF	0.05	0	0.12	0.00	**0.14**	0.00
lake area	-0.13	0	0.00	-0.09	-0.05	0.01
watershed area	-0.02	0	0.00	**-0.22**	-0.03	**-0.16**
maximum depth	-0.05	-0.03	0.08	0.00	0.00	0.01
alkalinity	**0.17**	0	**0.31**	0.09	**0.31**	0.11
water color	-0.05	0.08	-0.06	0.08	0.00	**0.14**
Secchi depth	0.02	0.05	0.00	**-0.24**	-0.04	-0.06
NH_4 water	0.10	0	0.02	0.02	0.03	0.02
TP water	-0.05	**-0.56**	0.00	-0.03	-0.02	0.00
TP sediment	**-0.27**	0.11	**-0.22**	0.00	-0.12	0.13

Table 5: PLSR results (contribution effect and direction) for community structure by growth form as response: types (emergent, submersed, floating leaved, and free floating). Variation (R^2) and significance (alpha = 0.05) are reported for the first two components combined with most contributing (squared loading weight >14%) predictor variables in bold.

	% Submersed		**% Emergent**		**% Floating leaf**	
	Comp1	**Comp2**	**Comp1**	**Comp2**	**Comp1**	**Comp2**
R^2	50.06	71.61	39.45	56.39	60.67	82.2
P-value	0.00	0.08	0.01	0.127	0.001	0.08
Connectivity variables						
# inflow	0.04	0.00	0.00	0.02	-0.09	0.09
# outflow	0.12	0.00	-0.04	0.07	**-0.15**	0
lake chain location	0.00	-0.09	-0.08	0.19	**-0.14**	0.05
Lake and Landscape variables						
SDF	-0.01	0.00	0.04	0.00	0	0.03
lake area	0.04	-0.03	0.08	0.00	0	0.08
watershed area	0.00	**-0.15**	0.01	0.14	0	0.13
maximum depth	0.00	**-0.16**	0.00	0.23	-0.01	0.06
alkalinity	**0.51**	0.12	**-0.34**	0.08	**-0.34**	0
water color	0.00	**0.15**	-0.09	0.00	**-0.14**	**-0.35**
Secchi depth	0.12	-0.04	-0.12	0.05	-0.04	0.05
NH_4 water	0.08	0.00	-0.07	0.00	-0.04	0
TP water	0.03	**-0.19**	0.10	**0.13**	0.01	0.12
TP sediment	-0.04	0.05	0.02	-0.09	0.03	-0.03

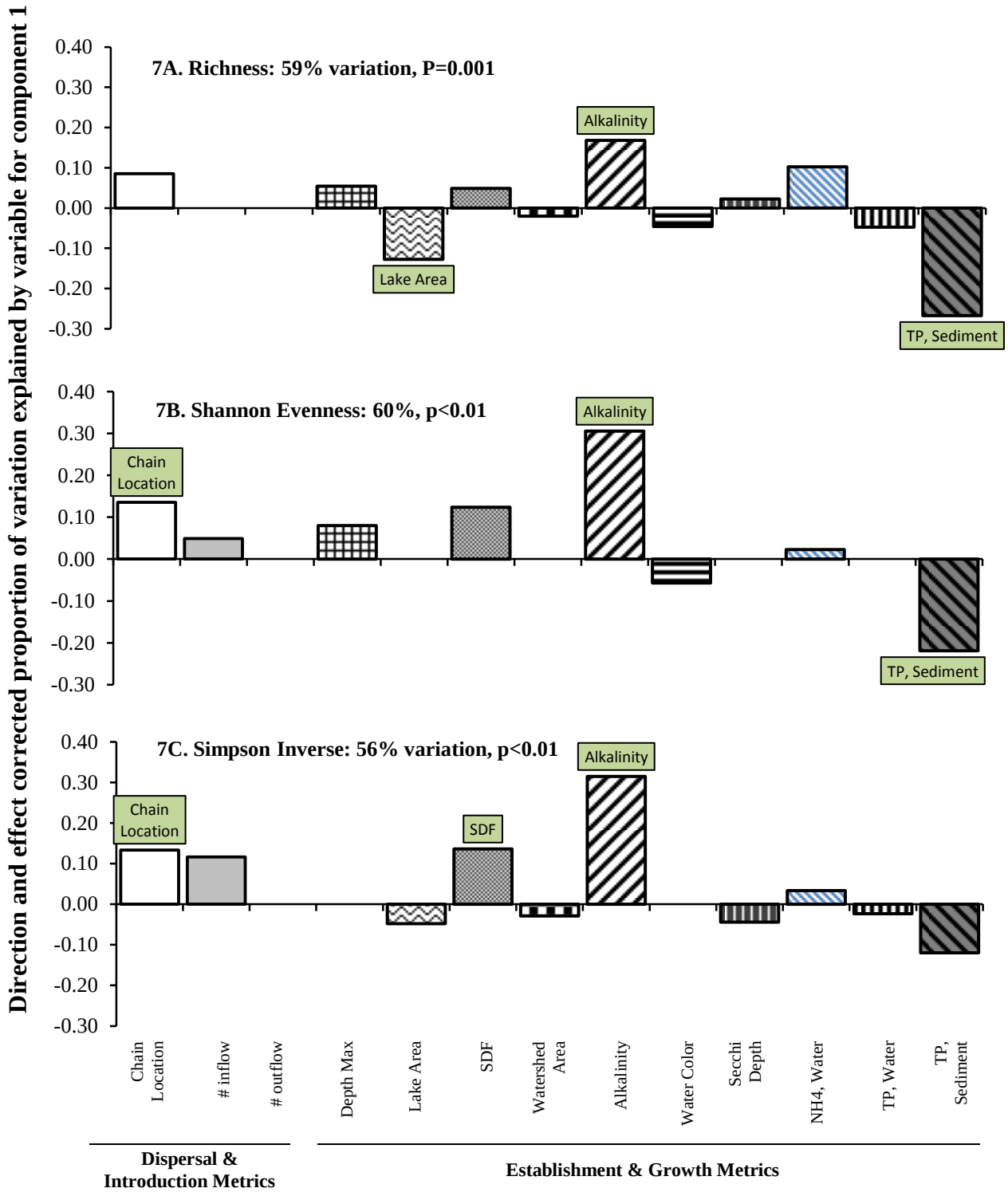

Figure 7: A. Richness, B. Shannon Evenness and, C. Inverse Simpson PLSR proportion plot corrected for effect and direction. The bar heights represent the strength of the variable and the location, above or below the axis line denotes direction (+/-) of the effect. The most important contributing (>0.14) variables are labeled with text. Variables are grouped with introduction and dispersal metrics on the left in solid colors and establishment and growth metrics are depicted with pattered bars.

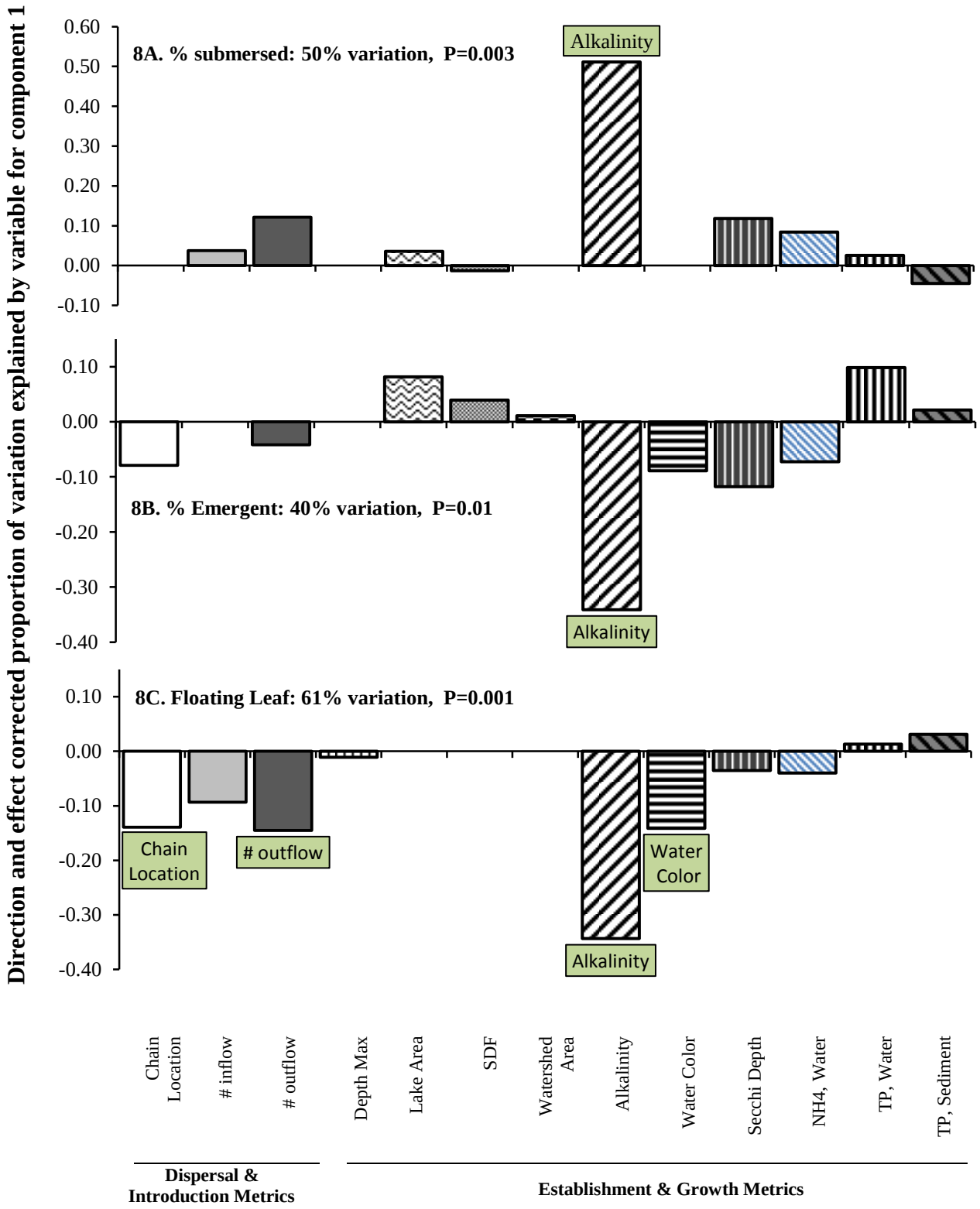

Figure 8: PLSR plots for community structure, growth form for A.submersed, B. emergent and C.floating-leaf growth forms.

DISCUSSION

Studies have shown that macrophyte community assemblages are shaped by a combination of factors that control the introduction/dispersal and establishment/growth of species across lakes (Dahlgren & Ehrlen 2005; Capers et al. 2009; Akasaka & Takamura 2011). However, these studies include exotic species, and few scientists have studied relatively undisturbed or protected systems to identify the factors influencing native macrophyte communities (Chambers et al. 2008; O'Hare et al. 2012). Our study of 'pristine' and native macrophyte communities on ISRO has provided two main insights about the relative roles of hydrological connectivity and lake and catchment characteristics in shaping macrophyte communities. First, we found that establishment and growth variables more often explained variation in macrophyte metrics than did introduction and dispersal variables. However, there are additional introduction/dispersal methods utilized by aquatic plants besides those that depend on hydrological connections (DeVlaming & Proctor 1968; Dalhgren & Ehlren 2005). Although measuring the effect of animal-mediated dispersal (i.e. moose or bird consumption and transport) of macrophyte species was beyond the scope of this study, these forms of movement may be important for macrophyte community composition. Thus, more information regarding macrophyte viability distances and fitness loss is needed for understanding the relative importance of such dispersal mechanisms.

Second, we found that there is no single predictor variable that drives all macrophyte response variables. This result suggests that identifying what drives macrophyte communities may depend on the macrophyte metric in question. Next, we describe the general results found for each macrophyte metric.

Drivers of species richness

When explaining variation in species richness, we found no introduction and dispersal variables to be significant. We expected that establishment and growth variables (i.e. SDF, lake area, lake depth, water clarity, lake nutrients) would have large positive influences on richness (Vesstergaard & Sand-Jenson 2000; Sondergaard et al. 2005). In fact, we found that richness increased with alkalinity and with decreasing sediment TP (Figure 7). These two associations have been found before and are not surprising, especially the relationship with alkalinity (Carpenter & Lodge 1986; Kalff 2001; Capers et al. 2009; Mikulyuk et al. 2010). Not only does alkalinity moderate and regulate fluctuations in pH, which contributes to a stabilized habitat suitable for a wider range of macrophytes, but alkalinity can directly influence bicarbonate levels, which can be utilized by some plants instead of CO^2 for completing metabolic processes (Kalff 2001; Kahara & Vermaat 2003; Capers et al. 2009).

Drivers of species diversity

We used two common diversity metrics, Shannon Evenness and Inverse Simpson Indeces, to characterize macrophyte community diversity. Similar to richness, both of these metrics responded to alkalinity and sediment TP (Figure 7), which are variables characterizing establishment. These findings are supported by previous work showing that macrophyte community diversity may be shaped mainly by water chemistry (Veestergaard & Sand-Jenson 2000; Søndergaard et al. 2005; Bronmark & Hansson 2005). Overall, neither diversity metric responded very strongly to connectivity nor dispersal-derived variables, although lake chain location did contribute to Shannon Evenness response. This particular finding could be attributed to the correlation between chemistry and lake chain position as chemistry has been known to be affected by the position of a lake in a lake chain (Soranno et al. 1999), with lakes

downstream or terminal within a chain containing higher levels of nutrients then upstream, origin lakes. This result could also be because these downstream lakes contain runoff from a higher area of the watershed then upstream, origin lakes. However, in our study, watershed size was not a significantly important contributing factor for the first component in any of our responses.

Drivers of community structure

We also found that community structure was influenced by different combinations of dispersal/introduction and establishment/growth variables. This result is not surprising since macrophyte growth forms have very different requirements and fill very different niches; therefore growth forms likely respond to different predictors (Alahuhta et al. 2013; Sousa, Thomaz & Murphy 2011;Akasaka & Takamura 2011, Heegaard et al. 2001).

The fact that submersed plants were mainly influenced by alkalinity (Figure 8) was expected because they grow entirely under the water surface and are sensitive to and rely on the chemistry composition of the water more so then other growth forms. (O'Hare et al. 2012; Bromark & Hansson 2005; Barko, Gunnison & Carpenter 1991). In contrast, it is estimated that the other growth forms that use both lake water and sediments for nutrients, as well as atmospheric CO_2, would be less dependent on lake chemistry (Akasaka & Takamura 2011; Kalff 2002).

Emergent species were primarily influenced by alkalinity and by Secchi depth (Figure 8). This result was expected because the majority of emergent species growth is above the water, and therefore these macrophytes are not reliant on water condition and are are less sensitive to water column measures of alkalinity or clarity (Kalff 2002; Alahuhta et al. 2013). However, the negative relationship with alkalinity was surprising. We hypothesize that this relationship is due to the negative correlation between proportion emergent and proportion submersed macrophytes.

Because submersed species make up the majority of species counted in these lakes (> 50%, n = 15), patterns seen with the other growth forms across all lakes will generally be similar, yet in contrast, to what were found for the submersed macrophytes.

Both emergent and floating-leaf species rely on a complex and heavy root system, and maintain most of their biomass above the water surface. Therefore, we expected similar results for these two growth forms. However, floating-leaf species responded to a combination of both dispersal and growth variables: floating-leaf species were negatively related to alkalinity, # connecting outflows, water color, and lake chain location. The strong negative relationship with alkalinity and water color may be explained by its strong negative correlation with submersed macrophytes, but the relationship with outflow and chain location is less clear. This result may be influenced by the area near outflowing aquatic connections being highly disturbed, resulting in high turbidity (measured by light attenuation of the water column), which is not good habitat for rooted and quiet-water species such as the majority of floating-leaf genera (Squires et al. 2002). Interestingly, our finding of floating leaf species decreasing as you progress down the lake chain is in contrast to the description of floating species as those that drift loosely and can easily 'float' with the current leaving and entering a lake (Sousa et al. 2011). However, we know that turbidity from both surface inflow and outflow connections increases with progression down a lake chain, which may negatively affect these species (Kratz et al. 1997; Soranno et al. 1999). However, our result will need to be tested further because we only sampled one three-lake lake chain (i.e., all but one lake chain included only two lakes).

Overall, we found that the introduction/dispersal and establishment/growth variables that shaped ISRO macrophyte community structure heavily depended on the growth form in question. This result makes sense because growth forms differ greatly in how they disperse and

reproduce (Dhlgren & Ehlren 2005; Sousa,Thomaz & Murphy 2011; Alahuntha et al. 2014). However, it was surprising that sediment TP was not a stronger correlate across all the growth forms. Because this variable was not shown to be a contributing factor to any growth form, this result suggests that while overall diversity and richness are related to sediment TP, phosphorous limitation is not specific to a particular growth form. This result could be more associated with rooting potential rather than growth type as suggested by Barko, Gunnison & Carpenter (1991), as submersed rooted species have been shown to rely on sediment phosphorous as much as 50% of total phosphorous intake, and non-rooted submersed species (e.g. *Utricularia spp.* aka bladderworts) can only utilize TP from the water column (Kalff 2002). Our findings demonstrate the importance of including growth forms, in addition to richness and diversity, as response variables when identifying drivers of macrophyte communities.

Surprising non-drivers of macrophyte communities

Some variables that we expected to be strongly associated with macrophyte richness, diversity, and growth form, such as Secchi disk depth, lake size, shape, and maximum lake depth (Hakanson 2005; Kalff 2002), were not consistently important predictors in our models. Next, we suggest some potential reasons for these results. Lake area or shape (as measured by SDF) was not a strong indicator of macrophyte richness, diversity, or community structure, although it has been shown in previous studies as being important to macrophyte communities (Squires et al. 2002; Hakanson 2005; Vesstergaard & Sand-Jenson 2000). Only Shannon evenness had a slight positive relationship with SDF, which is the pattern that is expected -- more irregularly shaped lakes should have more habitats available for species (Bronmark & Hansson 2005). However, richness was *negatively* related to lake area, which might show that while larger lakes can provide more available habitat for macrophytes to grow, they may also experience strong forces

of wave action and wind disturbance larger lakes, which can be detrimental to macrophyte growth (Sondergaard et al. 2005). Maximum depth was also not a significant factor for any macrophyte response metric although previous studies have shown that mean depth is related to macrophyte biomass (Squires et al. 2002). Perhaps this result is in part because the lakes on ISRO had a small range of lake depth, with them being mainly shallow lakes.

Other measures of diversity

Studies at the landscape-scale such as ours often make use of meta-community diversity metrics and quantify spatial autocorrelation of variables. Therefore, we conducted additional analyses to explore whether our results were being shaped by patterns at the meta-community scale or were driven solely by the distance among sample lakes. We calculated beta diversity for the 15 lakes sensu Legendre et al. (2005) and Akasaka & Takamura (2012).We used the additive and multiplative approach based on richness, where $\beta = \gamma - \alpha$ and $\beta = \gamma / \alpha$ respectively, where α = number of species per lake, γ = total number of species found across ISRO, and β = dissimilarity of each lake community to the entire ISRO community. Using these metric as a response variables in a PLSR model, we found that 64% of the variation was explained by the first component ($p = 0.0004$, Appendix 2, Figure A2). The results were very similar to those found for richness, with alkalinity strongly contributing to the observed variation, but in the opposite direction (Figure 7). We found almost identical results when we used the multiplative approach, with 69% of variation being explained by alkalinity and sediment TP ($p = 0.0001$; Appendix 2, Figure A2). These findings are not surprising because our measures of dissimilarity are derived from richness.

We calculated spatial autocorrelation with Moran's I Spatial Analysis tool in ArcGIS (version 10.1). None of the response variables or predictor variables that contributed to

component one for any model were spatially autocorrelated, indicating no spatial dependency. This result is likely because the lakes are relatively close to one another, remote from mainland influences, and are found in a relatively homogeneous landscape. In addition, these lakes share an island boundary, experience similar land use, and are all under the same management and protection status.

CONCLUSIONS AND IMPLICATIONS

Few macrophyte community studies have distinguished between dispersal/introduction (i.e. connectivity) and establishment/growth (i.e. lake and catchment) drivers of native macrophyte communities in protected and relatively undisturbed lakes such as those on ISRO. We measured macrophyte diversity three ways, as well as characterized three macrophyte growth form types, and three measures of hydrologic connectivity.

Generally, we found that 1) the drivers of macrophyte communities differed depending upon the macrophyte metric in question and 2) while the drivers of macrophyte communities included measures of both dispersal/introduction and establishment/growth, the most important and consistent drivers were metrics belonging to growth/establishment. Previously, we had little information about the role of hydrologic connectivity influencing macrophyte introduction and dispersal across lakes related to various macrophyte community metrics. By characterizing the relative roles of dispersal/introduction and establishment/growth on the ISRO native macrophyte diversity, we have supplied some important information that will help aquatic ecologists and park mangers to better-understand and recognize changes that may occur in the face of an invasive aquatic plant introduction.

Our study demonstrated that establishment/growth features, particularly chemistry variables such as alkalinity, were the strongest predictor associated with overall richness, diversity, and growth type. This result can be useful when predicting possible changes in response to invasive plant species introductions. Previous studies of north temperate lake systems have shown that invasive species richness was positively related to increased human activity and changes in pH (Capers et al. 2009). While human activity will remain at a minimum

on ISRO, pH can change depending on landscape and atmospheric inputs. It is therefore valuable that the information we have gathered regarding macrophyte relationships with alkalinity, which can directly influence and mediate changes in pH, is important for future monitoring the native plant communities of the island's inland lakes. Additionally, some of the establishment/growth variables that were important in our models, such as sediment nutrient concentration, alkalinity and water color are not included in some current lake monitoring programs and management plans yet are important to sample when monitoring lakes.

Our study demonstrates that lake position and the number of outflowing connections attached to a lake can influence the type of macrophytes growing in a lake, and particularly some rooted species. This information may be useful when predicting sites of likely establishment of invasive species that are introduced onto ISRO in the future. For example, we have documented where native emergents such as *Carex* sp. and native *Juncus spp.* are found on ISRO. Therefore, we can better predict likely establishment locations of the non-native narrow-leaf cattail, *Typha angustifolia* or exotic reed, *Phragmites australis*. Similarly, we can use information about where native milfoils are most successful on ISRO to predict where the submersed Eurasian Water milfoil, *Myriophylum spicatum*, might become established if it were introduced.

Previous surveys documented that ISRO lakes have some rare, endangered and special concern species of macrophytes (Voss and Reznick 2012). We found rare species such as *Myriophyllum farwellii* (Farwell's water-milfoil), *Myriophyllum alterniflorum* (alternate-leaf water milfoil), and *Sarracenia purpurea* (yellow pitcher plant). Although rare, these species can be found in at least one county within mainland Michigan or Wisconsin, suggesting that ISRO could provide restoration ecologists with native and rare plant seed banks that would be likely to be successful in the Great Lakes region, especially the Lake Superior Basin.

Our study provides some important baseline data about macrophyte diversity and community composition on ISRO that meets goals of the National Park Invasive Species Strategic plan (2008-2012). Specifically, this document calls for a 'Prevention, Early Detection and Eradication' research priority to 'Quantify genetic, ecological, and evolutional relationships among the species and ecosystems where they occur and…[the] ecological, social, and economic impacts of invasive species'. Our study has provided previously-undocumented macrophyte species lists for 15 ISRO lakes as well as information about what lake, catchment, and connectivity variables drive those macrophytes. It is imperative that scientists document reference aquatic plant distributions. This information provides important baseline data that we can use to compare anthropogenically disturbed systems to and to track responses to stressors over time, provide native community and species data, and to help us better-understand the implications of diversity and community structure changes.

APPENDICES

Appendix 1:

TABLES

Table A1: Species description and distribution per lake. Species list, including scientific name, common name, name CODE used for analysis simplicity, growth form type and total number of observations for all sampled 15 lakes (2012-2013) on ISRO. Riparian, moss and shrub species that were included within transect and abundance counts were not included in this list or macrophyte abundance estimates for diversity indices for this study as they are not defined by identification authority as aquatic macrophytes.

Scientific Name	Common Name	CODE	Growth Form	total No. of obs.	Ahmik	Angelworm	Beaver	Benson	Chickenbone	Desor	Feldtman	LeSage	Livermore	Mason	McDonald	Ojibway	Otter	Patterson	Richie
Bidens beckii	water merigold	BIDBEC	submersed	8	x		x		x			x	x		x		x	x	
Brasenia schreberi	water-shield	BRASCH	floating-leaf	1												x			
Carex spp.	sedge	CARSPP	emergent	6								x	x		x		x	x	x
Chara spp.	chara, muskgrass	CHASPP	submersed	11		x	x	x	x	x	x	x	x		x		x		x
Dulichium spp.	three-way sedge	DULSPP	emergent	2	x											x			
Elodia canadensis	common water-weed	ELOCAN	submersed	2						x									x
Eleocharis palustris	creeping spike-rush	ELEPAL	emergent	9	x		x		x		x			x		x	x	x	x
Eleocharis spp.	spike-rush	ELESPP	emergent	1														x	
Equisetum fluviatile	water-horsetail	EQUFLU	emergent	11		x	x	x	x	x		x	x		x		x	x	x
Eriocaulon aquaticum	pipewort	ERIAQU	submersed	8	x	x		x	x				x	x		x	x		
Glyceria borealis	northern mannagrass	GLYBOR	emergent	1		x													
Isoetes echinospora	Spiny-spore quillwort	ISOECH	submersed	9		x	x	x	x	x	x		x		x		x		
Isoetes lacustrus	lake quillwort	ISOLAC	submersed	2		x													x
Isoetes spp.	quillwort	ISOSPP	submersed	1						x									
Juncus spp.	water rush	JUNSPP	emergent	2							x	x							
Lemna triscula	star-duckweed	LEMTRI	submersed	1															x
Lobelia dortmanna	Water-lobelia	LOBDOR	submersed	3		x		x				x							
Myriophyllum alterniflorum	Alternate-flower water-milfoil	MYRALT	submersed	5	x				x					x	x				x
Myriophyllum farwellii	Farwell's water-milfoil	MYRFAR	submersed	1	x														
Myriophyllum sibiricum	common water-milfoil	MYRSIB	submersed	3					x								x		x
Myriophylum heterophylum	Various watr-milfoil	MYRHET	submersed	1											x				

Table A1 (cont'd)

Scientific Name	Common Name	CODE	Growth Form	total No. of obs.	Ahmik	Angelworm	Beaver	Benson	Chickenbone	Desor	Feldtman	LeSage	Livermore	Mason	McDonald	Ojibway	Otter	Patterson	Richie
Myriophyllum sp.	Water-milfoil	MYRSPP	submersed	7	x	x			x						x		x	x	x
Myriophyllum tenellum	creeping water-milfoil	MYRTEN	submersed	1							x								
Myriophyllum verticulatum	Whorled water-milfoil	MYRVER	submersed	3					x						x				x
Najas flexilis	northern water-nymph	NAJFLE	submersed	10	x	x	x	x	x	x		x	x				x		
Nuphar variegata	Bullhead lily, spadderdock	NUPVAR	floating-leaf	12	x	x	x	x	x		x	x	x		x		x	x	x
Nymphaea odorata	White water-lily	NYMODO	floating-leaf	5		x		x		x						x	x		
Phragmites australis	common reed	PHRAUS	emergent	3	x											x		x	
Potomogeton alpinus	Spotted pondweed	POTALP	submersed	1						x									
Potamogeton amplifolius	Bigleaf pondweed	POTAMP	submersed	7	x	x	x	x			x				x			x	
Potamogeton epihydrus	Ribbonleaf-pondweed	POTEPI	submersed	5	x	x		x		x					x				
Potamogeton foliosus	Leafy pondweed	POTFOL	submersed	1	x														
Potamogeton gramineus	Variable pondweed	POTGRA	submersed	14	x	x	x	x	x	x	x	x	x	x	x		x	x	x
Potomogeton hilli	Hill's pondweed	POTHIL	submersed	2											x		x		
Potamogeton natans	Floating pondweed	POTNAT	submersed	3				x			x					x			
Potamogeton obtusifolius	Bluntleaf-pondweed	POTOBT	submersed	1													x		
Potamogeton praelongus	Whitestem-pondweed	POTPRA	submersed	2	x							x							
Potamogeton pusillus	Small pondweed	POTPUS	submersed	1	x														
Potamogeton richardsonii	Clasping-leaved pondweed	POTRIC	submersed	6	x		x		x				x		x				x
Potamogeton robbinsii	Fern-pondweed	POTROB	submersed	2													x		x
Potamogeton spp.	pondweed	POTSPP	submersed	2											x			x	
Potomogeton spirillus	spiral pondweed	POTSPI	submersed	4		x		x	x						x				
Potamogeton zosteriformis	Flatstem-pondweed	POTZOS	submersed	10	x	x	x	x	x				x		x		x	x	x
Ranunculus flabellaris	Yellow water-crowfoot	RANFLA	submersed	1													x		
Ranunculus longiristris	Water buttercup	RANLON	submersed	1															x
Ranunculus trichophyllus	thread-leaf crowfoot	RANTRI	submersed	4	x				x				x		x				

Table A1 (cont'd)

Scientific Name	Common Name	CODE	Growth Form	total No. of obs.	Ahmik	Angelworm	Beaver	Benson	Chickenbone	Desor	Feldtman	LeSage	Livermore	Mason	McDonald	Ojibway	Otter	Patterson	Richie
Sagittaria cuneata	Arum-leaved arrowhead	SAGCUN	emergent	1												x			
Sagittaria latifolia	common arrowhead	SAGLAT	emergent	10	x		x		x	x		x	x	x	x		x	x	
Sagittaria spp.	arrowhead	SAGSPP	emergent	8		x	x	x				x	x	x			x		x
Schoenoplectus subterminalis	water-bulrush	SCHSUB	submersed	3		x		x						x					
Scirpis sp.	bulrush	SCISPP	submersed	1													x		
Sium suave	water-parsnip	SIUSUA	emergent	4					x			x		x				x	
Sparganium angustifolium	narrow-leaved bur-reed	SPAANG	emergent	1													x		
Sparganium fluctuans (Syn. Potamogeton pectinatus)	floating bur-reed	SPAFLU	emergent	4	x		x				x							x	
Sparganium spp.	bur-reed	SPASPP	emergent	5								x	x		x		x		x
Typha latifolia	common cat tail	TYPLAT	emergent	1															x
Stukenia filiformis	fineleaf pondweed	STUFIL	submersed	2						x		x							
Utricularia gibba	Creeping bladderwort	UTRGIB	submersed	1														x	
Utricularia intermedia	northern bladderwort	UTRINT	submersed	9	x			x				x	x	x	x	x	x	x	
Utricularia minor	lesser bladderwort	UTRMIN	submersed	6	x		x	x			x			x				x	
Utricularia vulgaris	common bladderwort	UTRVUL	submersed	9		x			x			x	x	x	x	x	x	x	
Utricularia spp.	unidentified bladderwort	UTRSPP	submersed	1										x					
Vallisneria americana	tape-grass, water celery	VALAME	submersed	1															x

Table A2: Percentages of aquatic macrophyte amounts categorized by growth type, listed in descending order by richness.

Lake	Richness	% Submersed	% Emergent	% Floating leaf
McDonald	24	79	17	4
Otter	23	65	26	9
Ahmik	22	77	18	5
Angelworm	22	68	23	9
Chickenbone	20	75	20	5
Benson	19	74	16	11
Richie	19	68	26	5
Patterson	18	61	33	6
LeSage	17	53	41	6
Livermore	16	69	25	6
Beaver	14	64	29	7
Mason	13	69	31	0
Feldtman	12	67	25	8
Desor	11	73	18	9
Ojibway	9	44	33	22

Table A3: Species abundance per lake with descriptive statistics for each species including sum relative abundance, mean relative abundance, maximum and min abundance and standard deviation (SD) for all lakes and total number of observations. Discrepancies between the species list within this table and Table A1 may occur as this table contains species identified within sample transects and may not reflect species observed outside of those sample transects.

Species Name (CODE)	Ahmik	Angelworm	Beaver	Benson	Chickenbone	Desor	Feldtman	LeSage	Livermore	Mason	McDonald	Ojibway	Otter	Patterson	Richie	SUM	MEAN	MAX	MIN	SD	No. occurrences
BIDBEC	0.25		0.03		0.07				0.04	0.05	0.14		0.06	0.04		0.67	**0.05**	0.25	0	0.07	8
BRASCH												0.53				0.53	**0.04**	0.53	0	0.14	1
CARSPP	0.09	0.01	0.14	0.05	0.06			0.06	0.19	0.06	0.14	0.12	0.03	0.31	0.01	1.28	**0.09**	0.31	0	0.08	13
CHASPP		0.16	0.03	0.02	0.17	0.15	0.10		0.09		0.08		0.21		0.01	1.01	**0.07**	0.21	0	0.07	10
DULSPP	0.02			0.01					0.00	0.01		0.05	0.02			0.11	**0.01**	0.05	0	0.01	6
ELOCAN															0.03	0.03	**0.00**	0.03	0	0.01	1
ELEPAL		0.01					0.01					0.00			0.07	0.09	**0.01**	0.07	0	0.02	4
ELESPP	0.09	0.01	0.05		0.08					0.10	0.05			0.01	0.02	0.41	**0.03**	0.10	0	0.04	8
EQUFLU	0.02		0.03	0.01	0.18	0.04			0.04	0.01	0.10		0.02	0.05	0.04	0.54	**0.04**	0.18	0	0.05	11
EQUSPP	0.01							0.01								0.02	**0.00**	0.01	0	0.00	2
ERIAQU		0.06		0.04	0.02			0.07		0.02			0.01			0.22	**0.01**	0.07	0	0.03	6
FONSPP				0.00								0.01				0.01	**0.00**	0.01	0	0.00	2
GLYBOR			0.01									0.01				0.02	**0.00**	0.01	0	0.00	2
ISOLAC		0.03											0.03			0.05	**0.00**	0.03	0	0.01	2
ISOSPP		0.31	0.09		0.04	0.08	0.01		0.02	0.05			0.15		0.18	0.92	**0.06**	0.31	0	0.09	9
JUNSPP							0.07									0.07	**0.01**	0.07	0	0.02	1
LEMTRI									0.01		0.01				0.24	0.26	**0.02**	0.24	0	0.06	3
LOBDOR		0.17						0.44								0.62	**0.04**	0.44	0	0.12	2
MYRALT					0.02						0.01		0.00			0.03	**0.00**	0.02	0	0.01	3
MYRFAR											0.01				0.01	0.02	**0.00**	0.01	0	0.00	2
MYRSIB					0.04						0.17		0.04	0.03		0.28	**0.02**	0.17	0	0.04	4
MYRSPP	0.02									0.00					0.02	0.04	**0.00**	0.02	0	0.01	3

Table A3 (cont'd)

Species Name	Ahmik	Angelworm	Beaver	Benson	Chickenbone	Desor	Feldtman	LeSage	Livermore	Mason	McDonald	Ojibway	Otter	Patterson	Richie	SUM	MEAN	MAX	MIN	SD	No. occurrences
MYRTEN							0.76									0.76	**0.05**	0.76	0	0.20	1
NAJFLE		0.06		0.24	0.09	0.14			0.06				0.06			0.65	**0.04**	0.24	0	0.07	6
NITSPP													0.01			0.01	**0.00**	0.01	0	0.00	1
NUPVAR	0.03		0.00		0.03			0.02		0.02		0.06		0.01		0.17	**0.01**	0.06	0	0.02	7
NYMODO				0.02		0.05						0.02				0.09	**0.01**	0.05	0	0.01	3
PHRAUS	0.02											0.02		0.08		0.11	**0.01**	0.08	0	0.02	3
SARPUR												0.00				0.00	**0.00**	0.00	0	0.00	1
POTAMP	0.06	0.01		0.05										0.04		0.16	**0.01**	0.06	0	0.02	4
POTEPI											0.02					0.02	**0.00**	0.02	0	0.00	1
POTFOL													0.04			0.04	**0.00**	0.04	0	0.01	1
POTGRA	0.02	0.02	0.02	0.05	0.04		0.03		0.05	0.01			0.17	0.00	0.03	0.44	**0.03**	0.17	0	0.04	11
POTNAT								0.01				0.01				0.02	**0.00**	0.01	0	0.00	2
POTOBT		0.01														0.01	**0.00**	0.01	0	0.00	1
POTPRA			0.18					0.06			0.20					0.45	**0.03**	0.20	0	0.07	3
POTPUS					0.01				0.02		0.03					0.06	**0.00**	0.03	0	0.01	3
POTRIC					0.04		0.00		0.09						0.13	0.27	**0.02**	0.13	0	0.04	4
POTROB													0.12		0.13	0.25	**0.02**	0.13	0	0.04	2
POTSPP		0.05				0.38	0.01									0.44	**0.03**	0.38	0	0.10	3
POTZOS	0.11		0.34			0.01			0.39				0.02		0.05	0.92	**0.06**	0.39	0	0.13	6
RANSPP	0.03				0.02								0.01			0.06	**0.00**	0.03	0	0.01	3
SAGSPP	0.01		0.01					0.22		0.21			0.01	0.07		0.52	**0.04**	0.22	0	0.08	6
SCISUB		0.07		0.50	0.01					0.17			0.02			0.77	**0.05**	0.50	0	0.13	5
SIUSUA	0.02				0.01					0.01			0.00			0.05	**0.00**	0.02	0	0.01	4
SPAFLU	0.15		0.05		0.06				0.01	0.01	0.02		0.01	0.03		0.33	**0.02**	0.15	0	0.04	8
SPASPP	0.01			0.00						0.15						0.16	**0.01**	0.15	0	0.04	3
TYPLAT															0.01	0.01	**0.00**	0.01	0	0.00	1

Table A3 (cont'd)

Species Name	Ahmik	Angelworm	Beaver	Benson	Chickenbone	Desor	Feldtman	LeSage	Livermore	Mason	McDonald	Ojibway	Otter	Patterson	Richie	SUM	MEAN	MAX	MIN	SD	No. occurrences
UTRINT	0.02			0.01				0.04		0.03		0.02		0.06		0.17	**0.01**	0.06	0	0.02	6
UTRMIN			0.00									0.00		0.01		0.01	**0.00**	0.01	0	0.00	3
UTRVUL	0.04		0.00	0.01	0.01			0.04		0.07	0.03	0.03		0.09		0.31	**0.02**	0.09	0	0.03	9
UTRSPP							0.00			0.02						0.02	**0.00**	0.02	0	0.00	2
VALAME															0.02	0.02	**0.00**	0.02	0	0.00	1
OTHER		0.00						0.03								0.03	**0.00**	0.03	0	0.01	2
VIBSPP												0.12		0.09		0.22	**0.01**	0.12	0	0.04	2
ALNSPP														0.09		0.09	**0.01**	0.09	0	0.02	1

Table A4: Using CARET package in R, the following predictors were identified as highly correlated (Pearson) at the absolute value of r = 0.60, 0.75 and 0.80 level. This information was used to identify which predictors could be removed from statistical analysis due to being highly correlated. Bold parameters indicate those that were shown correlated at all three tested levels and the most likely candidate for removal from modeling. The variables removed from the PLSR model will be lake perimeter, connectivity type and sediment alkalinity ($r > 0.80$, $p < 0.001$), in addition NH_4^+ sediment will be removed as it's highly correlated ($r = 0.77$, $p < 0.001$) with water NH_4^+.

	Pearson's Correlation where r =		
Parameter	**0.6**	**0.75**	**0.8**
Lake Area	X	X	
Lake Perimeter	X	X	X
Watershed Area	X	X	
Depth Max			
# inflow			
# outflow			
Connectivity type	X	X	X
Chain Location			
Alkalinity	X	X	X
Alkalinity sediment			
Water Color			
Secchi Depth	X		
NH_4^+, water			
NH_4^+, sediment	X	X	
TP, water			
TP, sediment	X		

Table A5: Specific variables identified in CARET analysis, their Pearson correlation coefficient (r, absolute value) and corresponding P-value to demonstrate the significance of their correlation and need to be dropped from further statistical analysis.

r <0.80

Variable X	Y	r	P
Lake Perimeter	Lake Area	0.865024	1.65127E-05
Watershed Area	Lake Perimeter	0.837829	5.7359E-05
Depth Max	perimeter	0.838039	5.68622E-05
Chain Location	Connectivity type	0.845154	4.20138E-05
Alkalinity, sediment	Alkalinity, water	0.858982	2.22711E-05

r< 0.75

Variable X	Y	r	P
Depth Max	Lake Area	0.77343	0.000517618
Depth Max	Watershed Area	0.771255	0.000550365
NH_4^+, sediment	NH_4^+, water	0.77395	0.000510042

R<0.65

Variable X	Y	r	P
Watershed Area	Lake Area	0.636919	0.009368545
# inflow	Lake Perimeter	0.663465	0.005998304
Secchi depth	# outflow	0.697403	0.003167337
TP, sediment	Water Color	0.658231	0.006572036

Appendix 2:

FIGURES

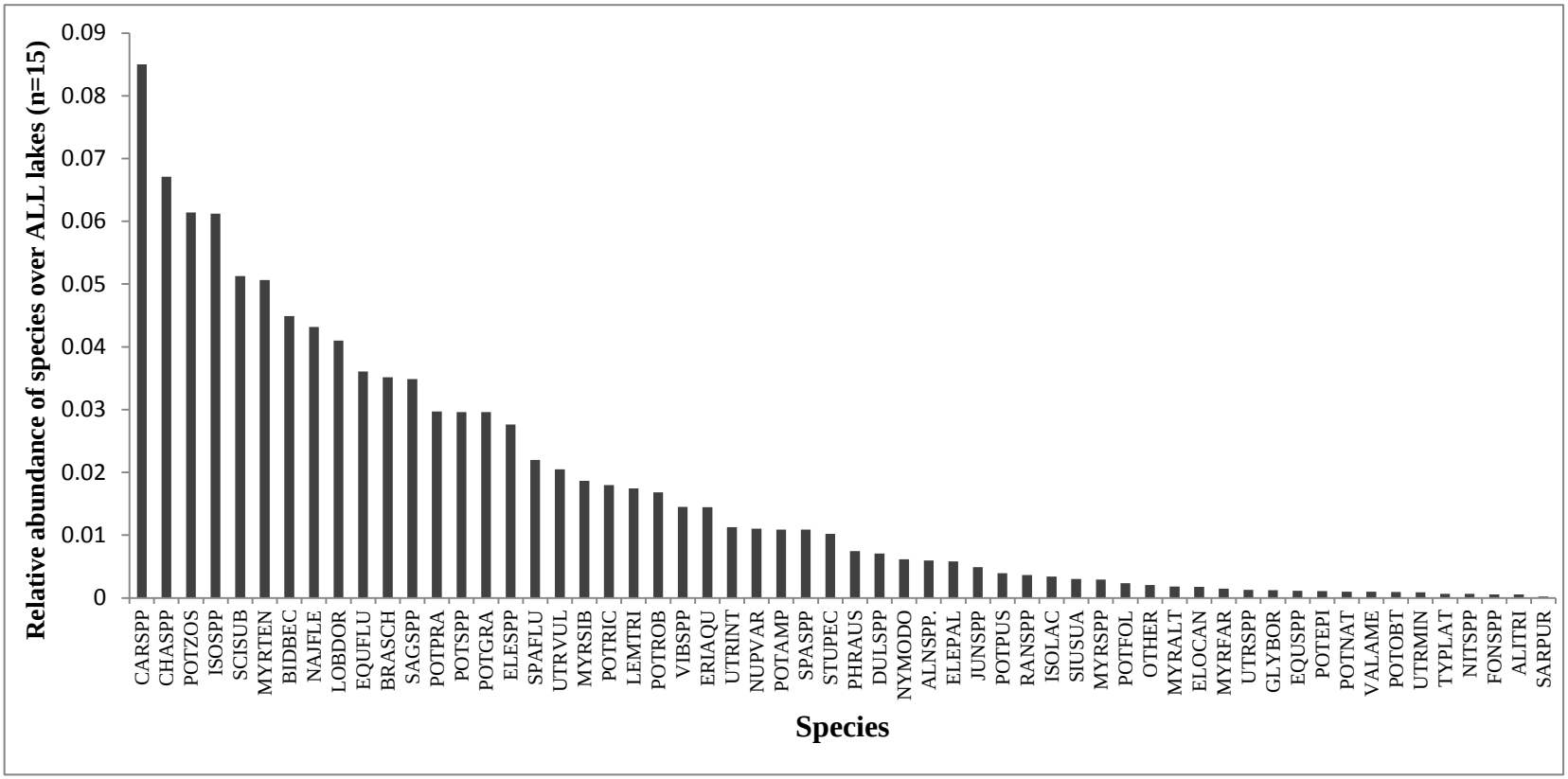

Figure A1: Relative abundances of each species, listed by species code, over all sample lakes (n=15).

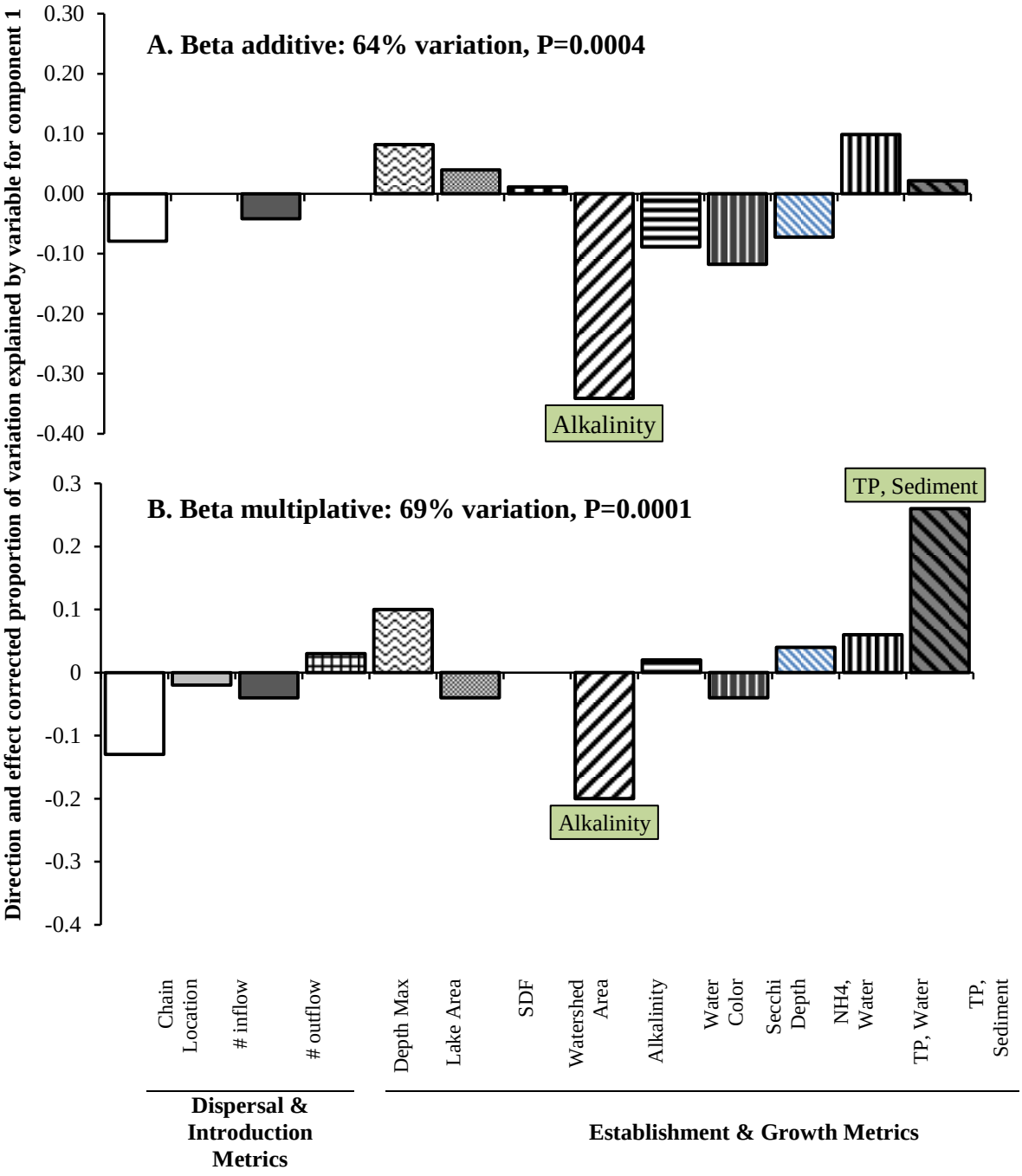

Figure A2: A. Beta diversity proportion plot using additive approach and B. Beta diversity proportion plot using multiplative approach.

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