Aquatic Epiphytes as Bioindicators of Wetland Health: A Study From The Simcoe County Wetlands

A thesis presented to
The Faculty of Graduate Studies
of
Lakehead University
by
Kate Read-Maney

In partial fulfillment of requirements
for the degree of
Master of Science in Biology
January 2026

Abstract

Aquatic microscopic epiphytes are increasingly recognized as sensitive bioindicators of aquatic ecosystem health, yet significant knowledge gaps remain regarding their indicator potential within Canadian wetlands. This study investigates the variability and environmental responsiveness of epiphytic communities to water quality changes as well as effects of the host species and structure. Two common macrophytes were used in this study - *Typha angustifolia* (alive and dead samples) and *Nymphaea odorata*. Four wetlands were studied, all from within the Lake Simcoe watershed (Ontario, Canada) - Langman's Marsh, Lagoon City, Holland Marsh, and Victoria Point. Three major gaps motivated this research: the scarcity of Canadian based epiphyte bioindicator research, the limited use of multi-genera epiphyte assemblages as bioindicators rather than single taxa, and the lack of understanding of host - epiphyte relationships in Canadian wetlands. Epiphyte samples were collected seasonally (Summer 2021, Fall 2021, Spring 2022). Standardized scraping, centrifugation, and microscopic analyses with a haemocytometer, were used to quantify epiphyte density, species richness, and diversity. Measurements were taken of phytoplankton communities, water chemistry (e.g., nutrients, DOC, chlorophyll-a), and in-situ parameters (e.g., DO, pH, conductivity) were obtained to evaluate and compare wetland health. Statistical analyses - including Shannon diversity indices, data transformations, and diagnostic modelling - were used to assess spatial and temporal patterns and to determine relationships between epiphyte assemblages, macrophyte hosts, seasons, and wetland conditions. Across sites and seasons, epiphyte communities demonstrated clear, measurable variation associated with macrophyte type, season, and wetland characteristics. Preliminary findings indicate that (1) epiphyte assemblages respond sensitively to environmental gradients, supporting their use as indicators of wetland health in Canadian wetlands; (2) host macrophytes influence epiphyte density and composition, with notable differences between senescent and alive macrophytes; and (3) wetlands with higher anthropogenic stressors exhibited distinct epiphyte community structures compared with less impacted sites. Importantly, several key genera - such as *Navicula* spp.*, Nitzschia* spp.*, Eunotia* spp.*, Cymbella* spp.*,* and *Gomphonema* spp., occurred across all wetlands, but their relative abundances differentiated stressed systems from healthier ones: wetlands where Navicula spp. and Nitzschia spp. dominated reflected nutrient pressure, whereas sites where these taxa coexisted alongside diverse, well-represented assemblages indicated more stable ecological conditions. Overall, this research provides a multi-wetland, multi-host assessment of aquatic microscopic epiphytes within the Lake Simcoe region and offers strong evidence supporting their utility as bioindicators in Canadian freshwater wetlands. The findings expand baseline ecological knowledge and contribute a valuable framework for future monitoring and conservation initiatives.

Acknowledgments

I would like to express my deepest gratitude to Dr. Nanda Kanavillil for his unwavering support throughout this rollercoaster of a thesis journey. From beginning during the COVID shutdowns to completing it after welcoming my third child to the world, the road was anything but straightforward. Nanda, your patience, encouragement, and belief in me, has made all the difference. I am forever grateful.

To my lab advisors, Vicki and Usha, thank you for opening my eyes to the beauty of teaching, learning, and leadership. Your guidance has shaped my research and inspired me as both a person and educator. I'll always cherish the laughter, camaraderie, and shared curiosity that made every challenge in the lab worthwhile.

Thank you to Lakehead University Orillia for the flexibility to pause my studies for health and family, and to the Orillia Fish & Game Conservation Club for sponsoring the microscope that allowed me to work from home.

And last, but certainly not least, to my family and friends. Thank you for never letting me quit on myself when things were hard. Thank you for never telling me to take the easy route and for always standing behind my academic aspirations. Your love and belief in me have been my greatest motivation, and I share this accomplishment with you.

Land Acknowledgment: This research was conducted on the beautiful traditional lands of the First Nation territory of the Anishinaabeg, specifically the Chippewas of Rama First Nation, Ojibway/Chippewa Odawa and Potawatomi Nations, members of the Chippewa Tri-Council and Three Fires Confederacy, and it continues to be home to many diverse First Nations, Metis and Inuit Peoples.

Layperson Summary

Wetlands are important ecosystems that help filter water, protect against floods, support different stages in wildlife life cycles, and increase biodiversity. In the water, on the surfaces of wetland plants, microscopic organisms called epiphytes grow. Although these microscopic communities can offer early warnings about changes in water quality, there is very little research about how they behave in Canadian wetlands. This study looks at the microscopic aquatic epiphytes growing on two common wetland plants, cattails (Typha angustifolia) and water lilies (Nymphaea odorata), across four wetlands in the Lake Simcoe region of Ontario: Langman's Marsh, Lagoon City, Holland Marsh, and Victoria Point.

By collecting plant samples during different seasons and examining the algae under a microscope, the research measured how many epiphytes were present, how diverse they were, and how their communities changed over seasons and between different wetlands. Water conditions such as dissolved oxygen, pH, and nutrient levels were also measured to understand the health of each wetland and what environmental factors might influence epiphyte growth.

The findings showed that epiphyte communities changed depending on the season, plant type, and water conditions at each wetland. Although some common epiphyte groups were found everywhere, their relative abundance - how dominant they were - told a much clearer story about wetland health. Wetlands under more human impact tended to be dominated by algae that thrive in nutrient rich or disturbed environments, while healthier wetlands had some of these same species but also many others present in stronger, more balanced numbers. These patterns show that epiphytes react quickly to environmental changes and can provide early, reliable signals of water quality.

Overall, this research helps fill a major knowledge gap by providing new information about epiphytes in Canadian freshwater environments and the effects of their host plants, as well as offers strong support for using these microorganisms to monitor and protect Canadian wetlands in the future

Table of Contents

List of Figures

List of Tables

Abbreviations

DO	Dissolved Oxygen
DOC	Dissolved Organic Carbon
HM	Holland Marsh
LC	Lagoon City
LM	Langman's Marsh
NO	*Nymphae odorata*
TA	*Typha angustifolia*
TAD	*Typha angustifolia* - Dead
TN	Total Nitrogen
TP	Total Phosphorus
VP	Victoria Point

Chapter 1 : Introduction

1.1 Background Research

Epiphytes, Phytoplankton, and Macrophytes

Microscopic epiphyte ecology encompasses a wide range of topics, including phylogeny, ecosystem roles, responses to environmental change, functional traits, genetics, seasonality, and interactions with macrophyte hosts. The following section summarizes key areas relevant to microscopic epiphytes and highlights the complexity of the field. This research focuses on freshwater microscopic epiphytes in Simcoe Muskoka wetlands. Much of the available literature focuses outside of Canada and often is on specific epiphyte species. The present review synthesizes information across all available freshwater algal epiphytes (excluding bacteria).

Epiphytes are increasingly recognized as effective bioindicators in wetland monitoring programs. Recent studies show that epiphytic algal communities respond rapidly to nutrient enrichment, turbidity changes, etc., making them reliable early warning indicators of declining water quality (Guiry et al., 2022; Laugaste et al., 2021). Wetlands support high numbers of species at risk and provide essential ecosystem services, a few examples include creating a habitat for a significant number of species, water purification, and flood regulation (UNEP, 2020). Because epiphytes integrate short- and long-term environmental signals, they are particularly useful for assessing ecosystem degradation and tracking restoration progress (Zhang et al., 2021).

Algae inhabit nearly all aquatic environments and even occur in extreme habitats such as thermal springs, snow, and hypersaline lakes (Wehr et al., 2015). Their ecological and functional diversity has expanded research into applications such as wastewater treatment, bioremediation, biofuel production, and so on (Nuhma et al., 2021). As algal biodiversity continues to be

documented, additional uses in environmental monitoring and ecosystem management are likely to emerge.

Epiphytes

Epiphytes are organisms that grow on plant surfaces without extracting nutrients from the host; they obtain water and nutrients from the environment and are not parasitic (Mondragón & Ticktin, 2015; Migiro, 2019). This research focuses specifically on microscopic aquatic epiphytes attached to submerged or emergent macrophytes.

Macrophytes

Macrophytes are aquatic plants that may be submerged, emergent, or floating, such as *Typha* spp., *Lemna* spp., and *Hydrilla verticillata*. They strongly influence habitat structure, nutrient cycling, and light availability, in wetlands and shallow lakes (U.S. EPA, 2020). Their surfaces provide a stable substrate for epiphytic algae.

Phytoplankton, Periphyton, and Epiphyton

Phytoplankton are free-floating microscopic algae found in the sunlit layers of marine and freshwater ecosystems, dominated by cyanobacteria, green algae, diatoms, and flagellates in North American lakes (U.S. EPA, 2019). Periphyton refers to algae attached to hard substrates such as rocks or sediment (Likens, 2009). When algae attach specifically to macrophyte surfaces, are referred to as epiphytes. Some phytoplankton can transition into epiphytic forms when surfaces become available, and epiphytes can re-enter the planktonic community following hydrodynamic disturbance (Zadorozhna et al., 2017).

Algae lack roots, stems, and vascular tissues and may be unicellular, colonial, or filamentous (Vidyasagar, 2016; Wehr et al., 2015). They contribute significantly to global carbon cycling and oxygen production; diatoms alone produce an estimated 20-25% of global oxygen

(Benoiston et al., 2017; Singh, 2020). Algal community composition varies with nutrient levels, light, hydrology, and season (Wehr et al., 2015). Their taxonomy remains complex because algae represent multiple evolutionary lineages across different kingdoms, and modern classifications use pigment composition, storage products, flagellar structure, and so on (Wehr et al., 2015).

Phytoplankton - Epiphyton Relationship

A study in 2017 found that epiphytic algae and phytoplankton exhibit an exchange, with species moving between communities in response to disturbance, hydrology, and nutrient conditions (Zhang, et al., 2021; Cantonati et al., 2017). Phytoplankton typically experience higher light and suspended nutrient levels, while epiphytes benefit from substrate stability, lower grazing pressure, and access to macrophyte microhabitats.

Light availability and nutrient enrichment are major drivers of competition between phytoplankton and epiphytes. Elevated nutrient levels can create rapid phytoplankton growth, and as these populations expand, they reduce light penetration in the water column. This shading effect suppresses epiphytic algae population growth, shifting the competitive balance toward phytoplankton dominance (Wang et al., 2024).

Attachment Mechanisms of Microscopic Epiphytes

Different groups of microscopic algae employ diverse attachment mechanisms. Diatoms, which dominate many epiphytic communities, may attach using extracellular polymeric substances, mucilage pads, stalks, or prostrate adhesion depending on flow conditions and substrate texture (Kanavillil et al., 2014; Kanavillil & Takada, 2024). Attachment is influenced by surface roughness, with rougher surfaces providing more microhabitats for adhesion (Proaño Peña et al., 2023). A study on early biofilm formation demonstrated that diatom species show preference for certain microhabitats on substrates, with species richness differing between center

and edge zones of glass slides (Kanavillil et al., 2014).

Diatoms

Diatoms derive their name from a Greek term meaning “cut in half,” reflecting the two-part silica frustule (Jewson & Granum, 2019). Frustule morphology is essential for species identification and reflects extensive evolutionary diversification. An estimated 200,000 species exist globally (Guiry & Guiry, 2023). Diatoms inhabit nearly all aquatic ecosystems and often dominate freshwater habitats (Wehr et al., 2015). They contribute to approximately 20% of all global carbon fixation (Hildebrand et al., 2020; Benoiston et al., 2017) and play crucial roles in biogeochemical cycles. They may be planktonic, benthic, epiphytic, solitary, or colonial.

Major Algal Group

Modern literature recognizes multiple major algal groups - including Cyanobacteria, Chlorophyta, Euglenophyta, Eustigmatophyceae, Chrysophyceae, Synurophyceae, Bacillariophyta (diatoms), Haptophyta, Dinophyceae, Cryptophyta, and Phaeophyceae (Figure 1.1). Each characterized by distinct pigment compositions, cell structures, and ecological roles. Many groups contain both microscopic and macroscopic representatives (Wehr et al., 2015; Guiry, 2023).

Algal group (Chapter Number)	Photosynthetic Pigments[b]	Chloroplast Outer Membranes	Thylakoid Associations	Starch-Like Reserve[c]	External Covering[d]	Flagella
Cyanobacteria (Chapters 3 and 4)	chl *a*, PE, PC, APC	0	0	Cyanophycean	Pepitoglycan matrices or walls	**0**
Red algae (Chapter 5)	chl *a*, PE, PC, APC	2	0	Floridean	Walls with galactose polymer matrix	0
Green algae (Chapters 6-9)	chl *a*, *b*	2	2-6	True	Cellulosic walls, scales	0-many
Euglenoid algae (Chapter 10)	chl *a*, *b*	3	3	Paramylon	Pellicle, lorica	1-2 emergent
Xanthophytes and Raphidophytes (Chapter 11)	chl *a*, c_1, c_2, diadinoxanthin, heteroxanthin, vaucheriaxanthin	4	3	Chrysolaminarin	Mostly cellulosic walls, some naked	2 unequal
Eustigmatophytes (Chapter 11)	chl *a*, violaxanthin, vaucheriaxanthin	4	3	Chrysolaminarin	Mostly cellulosic walls	1-2 (unequal)
Chrysophyte algae (Chapter 12)	chl *a*, c_1, c_2, c_3, fucoxanthin	4	3	Chrysolaminarin	None, scales, lorica	2 unequal
Haptophyte algae (Chapter 13)	chl *a*, c_1, c_2, c_3, fucoxanthin, diadinoxanthin,	4	3	Chrysolaminarin	Non-siliceous scales	2 equal + haptonema
Synurophyte algae (Chapter 14)	chl *a*, c_1, c_2, c_3, fucoxanthin	4	3	Chrysolaminarin	Siliceous scales	2 unequal
Diatoms (Chapters 15 and 16)	chl *a*, c_1, c_2, c_3, fucoxanthin, diatoxanthin, diadinoxanthin	4	3	Chrysolaminarin	Siliceous frustule	1 (only rarely)
Dinoflagellates (Chapter 17)	chl *a*, c_2, peridinin	3	3	True	Cellulosic theca	2 unequal
Cryptomonads (Chapter 18)	chl *a*, c_2, PC or PE, alloxnathin	4	2	True	Periplast	2 equal
Brown algae (Chapter 19)	chl *a*, c_1, c_2, c_3 fucoxanthin	4	3	Laminarin	Walls with alginate matrices	2 unequal: transverse + longitudinal

[a] *From various phycology textbooks (e.g., Graham et al., 2008; Lee, 2008).*
[b] *chl = chlorophyll (green), PE = phycoerythrin (red), PC = phycocyanin (blue), APC = allophycocyanin (blue), fucoxanthin and peridinin (golden to brown).*
[c] *All of the reserves are polymers of glucose; they differ by their linkages; cyanophycean and floridean α-1,4 and α-1,6 branches; true with amylose α-1, 4 and amylopectin α-1,4 and α-1,6 branches; paramylon β-1,3; chrysolaminarin and laminarin β-1,3 and β-1,6 branches. Only true starch stains positively with iodine (purple to black).*
[d] *Pellicle and periplast within plasma membrane; the rest are external to it.*

Figure 1.1 - The major distinguishing traits of the 13 major algal groups (Source: Freshwater Algae of North America, Wehr, J. H, 2015).

Reproduction in algae

Reproduction in algae is highly diverse, with most species reproducing asexually, or sexually through fragmentation or gamete production. Diatoms, however, exhibit a life cycle distinct from most algal groups, involving both sexual and asexual modes of reproduction (Figure 1). During asexual reproduction, the cell divides by separating its valves (frustules) and regenerating the missing half, producing two daughter cells - one larger and one smaller (Sánchez et al., 2019). Over successive divisions, this process leads to a progressive reduction in average cell size because the new wall elements (girdle and bands) are formed within the parent frustule.

Once a diatom reaches its minimum viable size, it can no longer continue asexual division and must restore its size either through sexual reproduction or vegetative enlargement (Jewson et al., 2022; Kaczmarska et al., 2013). Sexual reproduction involves the formation of an auxospore following the fusion of male and female gametes. Because the auxospore lacks a rigid silica wall, it can expand before forming a new full-sized frustule (Kale and Karthick, 2015). Alternatively, vegetative enlargement involves shedding the restrictive frustule, enlarging the protoplast, and then producing a new frustule (Kaczmarska et al., 2022).

Resting cells represent another important stage in diatom life cycles, activated only when environmental conditions become unfavorable. These cells exhibit reduced metabolic activity and play a key role in survival under fluctuating or extreme conditions (Balestra et al., 2021; Souffreau et al., 2013).

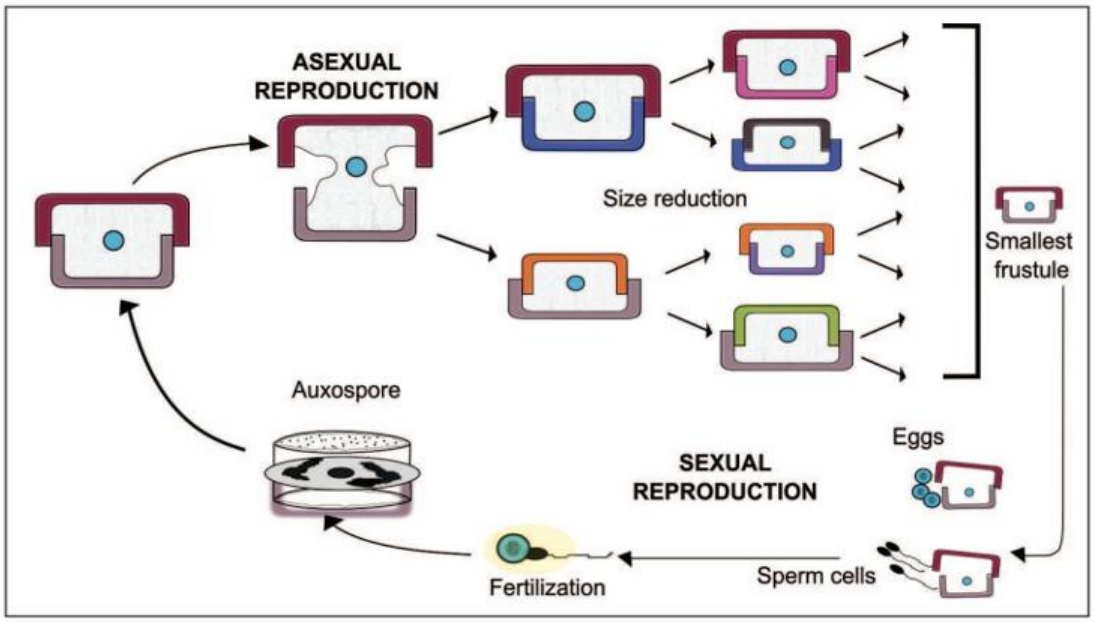


Figure 1.2 - An illustration of the life cycle of a diatom, including both sexual and asexual reproduction (Source: Kale and Karthick., 2015)

Aquatic Epiphytes in Research

Epiphytic algae, particularly diatoms, have been widely used in ecological research and biomonitoring. For example, epiphytic diatoms have been applied as indicators of ecological conditions in New Zealand's lowland wetlands (Cantonati, 2020; Kilroy et al., 2016), used as freshwater bioindicators in urban wetlands of Central Gujarat (Singh & Parikh, 2020), and assessed for their taxonomic composition and size distributions in relation to ecological and toxicological variables in Lake Saint François, Quebec (Cattaneo et al., 1995). Effective use of epiphytic diatoms in biomonitoring requires consideration of substrate specificity, species diversity, and accurate taxonomic identification (LetáKová et al., 2018).

The Epiphyte and Macrophyte Relationship

The relationship between macrophytes and their epiphytic algal communities varies widely depending on species traits and environmental conditions. Diatoms may occur as phytoplankton or as epiphytes, attaching to macrophyte surfaces using extracellular polymeric substances (EPS) (Xu et al., 2023; LetáKová et al., 2018). Because they attach only externally, they are not considered parasitic. Host plant surfaces excert both physical and chemical influences on diatom colonization (Pie et al., 2023). A 2023 study reported that plants free of epiphytes exhibited more shoots and stems with greater diameters, suggesting that epiphytes may alter host morphology (Somma et al., 2023).

Submerged macrophytes can shape epiphytic algal assemblages by modifying nutrient availability and light conditions (Zhang et al., 2021). A 2018 study in China demonstrated that epiphytic algal richness increased with greater macrophyte coverage, likely due to improved water transparency and regulated nutrient availability (Lv, 2019). While nutrients are essential for epiphyte growth, excessive nutrient levels can trigger eutrophication and negatively impact communities (Wilkinson, 2020).

Some macrophytes release allelopathic compounds that inhibit algal photosynthesis, reducing microalgal biomass (Hilt, 2006; Zhu, 2019). This interaction may reflect a coevolutionary "arms race" between macrophytes and epiphytes (Somma et al., 2023). In some cases, epiphytes benefit their macrophyte hosts; for instance, moderate shading by epiphytes may protect macrophytes from UV radiation (Klancnik, 2015), and epiphytes may serve as protective barriers against grazers and pathogens (Somma et al., 2023). Conversely, macrophytes benefit epiphytes by providing more stable attachment surfaces, access to light, and localized nutrient availability (LetáKová et al., 2018).

Extracellular Polymeric Substance (EPS)

EPS are secreted by microorganisms in aquatic and terrestrial biofilms and serve numerous critical ecological functions (Decho et al., 2017). These multi-functioning hydrated polymeric matrices consist primarily of polysaccharides, proteins, and other organic molecules. EPSs aid in surface adhesion, enhance protection from environmental stress, facilitate genetic exchange, support fertilization, provide antibiotic resistance, and help retain nutrients (Decho et al., 2017; Costa et al., 2018). Changes in environmental conditions - such as fluctuations in pH, temperature, or salinity - can stimulate EPS production (Flemming, 2019; Costa et al., 2018; Vardharajula et al., 2015). EPS also serve as an important carbon source for grazers and can bind metals and pesticides, influencing contaminant transfer through the food web (Decho et al., 2017).

Major Water Quality Parameters Impacting Epiphytes

Stormwater Runoff

Phosphorus is essential for biological productivity, but stormwater with excessive phosphorus content can lead to rapid algal and macrophyte growth and eventually reduced

dissolved oxygen due to decomposition (EPA, 2017). Major phosphorus sources include fertilizers, septic systems, and animal waste. Increasing urbanization reduces permeable land cover, resulting in greater stormwater runoff and higher nutrient loading into watersheds (LSRCA, 2018).

Conductivity

Conductivity reflects the concentration of dissolved inorganic ions (EPA, 2012). Diatom communities respond sensitively to conductivity gradients, with shifts from moderately tolerant species to highly pollution-tolerant taxa as conductivity increases (Mangadze, 2017). Bere et al., similarly observed that dissolved salt concentrations explained a substantial portion of variation in diatom assemblage structure (Bere, 2010; Angelis et al., 2020).

Water Temperature

Water temperature influences diatom growth, chemical processes, and metabolic rates (USGS, 2021). Hinz et al. found that although growth rates increased with temperature, each species exhibited an upper thermal threshold beyond which growth declined. They proposed that decreased water viscosity at higher temperatures may increase sinking rates, and reduced cell size may help counteract sinking by improving buoyancy (Hinz et al., 2018).

pH

pH strongly influences diatom distribution (Teittinen et al., 2021). Research on acidic environments shows sharp declines in species richness below pH 3.5, with most taxa restricted to narrow pH ranges (DeNicola, 2000; Teittinen et al., 2021). These patterns highlight the utility of diatoms as pH bioindicators. Recent studies further demonstrate predictable shifts in diatom assemblages across natural pH gradients, with acid-tolerant species dominating low pH waters

and more diverse communities thriving near neutral pH (Teittinen et al., 2021; Saros & Anderson, 2021).

Nutrient Content

Nitrogen and phosphorus are the primary limiting nutrients for aquatic primary producers. Excess nitrogen can trigger harmful algal blooms, while nitrogen depletion may reduce phytoplankton diversity (USGS, 2021c; TERC, 2021). Laboratory experiments show that diatom growth increases with elevated phosphate, silicate, and vitamin concentrations (Orefice et al., 2019; Yang et al., 2023, 2017; Bertrand & Saito, 2021). Diatoms have also been widely used to assess nutrient pollution, with assemblage changes reflecting nutrient enrichment in streams and rivers (Charles et al., 2018; Charles et al., 2019). Overall, diatoms respond rapidly to nutrient fluctuations, making them useful early-warning indicators of wetland health (Kilroy, 2017).

Other Pollutants

Pharmaceuticals and personal care products (PPCPs) - including detergents, antibiotics, hormones, and caffeine - enter aquatic systems primarily through wastewater effluents (Wydro, 2024). These contaminants have been shown to alter phytoplankton community structure and reduce epiphyte richness at higher concentrations (Wilson et al., 2003; Pinho et al., 2022; Santos et al., 2023).

Wetland Ecology

Wetlands are ecosystems characterized by saturated soils, organic matter accumulation, and vegetation adapted to inundated conditions (Mitsch & Gosselink, 2015). They are commonly classified as marshes, swamps, bogs, fens, or tidal wetlands (Junk, 2023; Sivaperuman, 2015; OntarioMNR, 2025). Canada contains approximately 25% of the world's wetlands, with Ontario possessing an estimated 35 million hectares (OntarioMNR, 2025). Global assessments identify

pollution, landscape conversion, biological resource use, and agriculture, as major threats to wetland health (Xu et al., 2019). Canada currently has 37 Ramsar designated wetlands of international importance, covering over 13 million hectares (Ramsar Canada, 2014).

Historically undervalued, wetlands are now recognized for their ecological and societal importance. They provide wildlife habitat, water filtration, flood mitigation, carbon storage, shoreline protection, and recreational opportunities (EPA, 2016). Southern Ontario has lost an estimated 72% of its wetlands to development since the 1800s (Kraus, 2019). Despite occupying relatively small areas, wetlands support disproportionately high biodiversity, including many at-risk species. They also play a key role in nutrient regulation between terrestrial and aquatic systems and serve as nurseries for numerous fish species (Mitsch & Gosselink, 2007).

Algae as Bioindicators

Algae are widely recognized as sensitive and effective indicators of environmental change due to their short life cycles and their rapid responses to fluctuations in nutrients, pH, temperature, and light (Bellinger & Sigee, 2015; Liu et al., 2021). Their broad spatial and temporal distributions, high diversity, and abundant populations enhance their utility in monitoring ecological health (Guo et al., 2022). Seasonal variations in phytoplankton communities have been linked to changing hydrographic and nutrient conditions (Kim, 2019).

Diatoms are widely applied as bioindicators of water quality impacts because of their high taxonomic diversity, variation in morphology, and their broad range of ecological preferences. There are two main forms of diatoms: centric and pennate. Centric diatoms are predominantly planktonic and are strongly influenced by water column conditions such as nutrient availability and mixing, with numerous lake studies showing increases in planktonic (and centric) diatoms under eutrophication conditions (Lobo et al, 2016; Rimet & Bouchez,

2012). Pennate diatoms are primarily benthic or attached to substrate and respond more directly to local water quality conditions, including nutrient enrichment, organic pollution, conductivity, and substrate characteristics (Masouras et al., 2021). Changes in the relative dominance of centric versus pennate diatoms are commonly interpreted as reflecting different types of water quality change rather than a uniform response to pollution. This change in ratio of centric and pennate diatoms has been successfully used in recent water quality research, specifically to the changes in total phosphorous in the water (Liu et al., 2020).

1.2 Research Objectives

Epiphyte ecology is an enormously broad subject with themes ranging from water quality impacts to genetic mutations. It encompasses a myriad of species and their life cycles. Research on epiphytes is ramping up throughout the world due to their usefulness as bioindicators and as well as their commercial uses as an agent of bioremediation, source of biofuels etc. Even though we have learned a lot about epiphytes over the last few decades, there are still gaps in many aspects of our understanding of them, especially from a Canadian perspective.

The three major knowledge gaps that are going to be addressed in this research are:

1) The lack of information on epiphytes as bioindicators from Canadian wetlands. Much of the research are from international wetlands.
2) There are not enough studies that use multiple epiphytes extracted from wetland macrophytes as bioindicators of wetland health. The majority of studies focus on one or two epiphyte species instead of looking at a community level.
3) Not enough is known about the possible relationships between the wetland macrophytes and their epiphytic microalgal community.

Filling of each of these gaps could be extremely useful in helping us better

understand the potential of use of epiphytes as bioindicators in Canadian environments. This research plans to approach the above-mentioned knowledge gaps by examining the microscopic epiphyte populations on selected wetland macrophytes and their usefulness to study the health of wetlands. This study will then determine if epiphytes can be used to indicate environmental variations. Previous studies have provided us with enough evidence that epiphytes react to the changes in their environment, depending on the species, and by identifying these changes, this study will be able to support and or deny the possibility of using epiphytes as environmental indicators of wetland water quality in Central Ontario, Canada. Thus, the objectives of this research are to:

1. Examine the microscopic aquatic epiphyte populations on selected wetland macrophytes
2. Identify any discernible patterns in the epiphyte communities with the wetlands, macrophytes and the seasons, and
3. To investigate reasons for any observed differences or similarities in epiphyte community, including water quality and trophic status.

This study will then determine if epiphytes can be used to indicate wetland water quality and thus the environmental impacts. We hypothesize that:

(1) microscopic epiphytes can be used as wetland health indicators,

(2) microscopic epiphyte population vary with macrophyte species due to variations in host epiphyte relationship, and

(3) epiphyte population on macrophytes vary between wetlands and the seasons.

Chapter 2 : Methods

2.1 Selection and Description of Sampling Locations

The wetlands sampled for this study were located in the Lake Simcoe watershed, Ontario, Canada. Lake Simcoe is the fourth largest lake in Ontario, with a surface area of 722 sq km and an average depth of 15 meters (some areas are as deep as 42 meters). The earliest known name of Lake Simcoe was Ouentironk (oo ent' er onk), the Huron word for "beautiful water." Lake Simcoe is home to 50 different fish species including the yellow perch, whitefish, and lake trout. Lake Simcoe is also home to invasive species, such as zebra mussels, Eurasian watermilfoil, and the common carp. There are approximately 32 species at risk found in the Lake Simcoe wetlands, including the southern flying squirrel, the eastern hog nose snake, the red shouldered hawk, and the Jefferson salamander (LSRCA, 2023).

Lake Simcoe is part of the Lake Simcoe Watershed, which comprises of 3400 square kilometres area, and 13% of this watershed is wetlands. This area hosts approximately 500,000 human residents, 75 fish species, and 18 major river systems. The Lake Simcoe watershed is a significant source of tourism income for the County, generating approximately $200 million annually, primarily from ice fishing tourism (LSRCA, 2023). There are 18 major river systems as well as other smaller streams, creeks, and tributaries flow into Lake Simcoe (LSRCA, 2023).

Provincially Significant Wetlands (PSW) are wetlands identified as being the most valuable ones (MNR, 2023). These rankings are determined by a point system formulated by the Ontario Ministry of Natural Resources. PSWs are described as having a score of a minimum of 600 points overall or a minimum of 200 points in biological component in the Ontario Wetland Evaluation System (OWES) (NHR, 2023). The four main components of the point system are biological, social, hydrological, and special features component. The biological component looks at the productivity and diversity of the habitat. The social component measures economical and

recreational values. The hydrological component considers water related values, including issues such as flooding, water quality, groundwater recharge, etc. The special features component recognizes the geography, rare species, ecosystem age, and habitat quality for the local wildlife. Each component has its own grading system, and all components are added together to reach the final grading score (MNR, 2023).

An essential part of water quality analysis is the measurement of phosphorus. It is a critical nutrient for all life, especially in the freshwater ecosystems; however, too much phosphorus can devastate lakes (USGS, 2023). This nutrient loading can cause rapid growth of algae and large macrophytes, which can eventually decrease the levels of dissolved oxygen in the water through the death and decomposition of the organic debris. A significant stressor on Lake Simcoe watershed health is the urban stormwater runoff, which accounts for ~ 31% of the phosphorus loading into Lake Simcoe (EPA, 2017). Some other major sources of phosphorus include farm fertilizer and septic system discharges.

The Lake Simcoe Region Conservation Authority (LSRCA), prepared a 2023 watershed report card, using data from 2017-2021, consisting of groundwater quality, surface water quality, forest conditions, and lake ice cover. The watershed grading is broken down to sub watershed sections, each with a grade of very poor, poor, fair, good, or excellent. Overall, the report was positive stating that Lake Simcoe is the cleanest lake in the Great Lakes region. The groundwater quality was measured by concentrations of nitrate, nitrite, and chloride. The Lake Simcoe Watershed was graded as having an excellent groundwater quality across the watershed, with only two (out of 13) wells tested receiving a lower grade. The forest conditions were measured by the forest cover percentages, forest interior, and streamside cover. The rating results varied by location from excellent to poor, with the majority rated fair or above. The forest conditions tend

to be lowered based on how the land is used in that sub-watershed, such as urban areas or agricultural uses lowering the land quality (LSRCA, 2023).

Ice coverage days in the Great Lakes have been depleting every year since the beginning of records. Lake ice coverage is beneficial to many different species and systems in the Great Lakes, including protecting the eggs of white fish or with reducing lake evaporation and stratification, as well as reducing runoff. Reduced lake ice coverage can increase nutrient loading, and as stated before, can be detrimental to the Lake's biotic components (GLISA, 2023).

Ice coverage helps to provide protection for seeding populations of phytoplankton. The lake phytoplankton populations shift later in the season as the grazing pressure increases, implying that the ice coverage reduces early season phytoplankton blooms from occurring (Hazuková, 2021). The control of phytoplankton communities is essential to keep the health of the lakes. Phytoplankton can also cause catastrophic effects on an aquatic ecosystem through dangerous blooms (cyanobacterial bloom) and the red tide (dinoflagellate bloom, less common in lakes) phenomenon (Chorus et al., 2021).

The lake ice cover for Lake Simcoe has drastically reduced since 1852, when the average period of ice cover in Kempenfelt Bay was around 126 days. Today that number is around 92 days. The likely prime suspect for this reduction is climate change. The winters are, on average, warmer, with fewer days below zero degree centigrade. Another factor is the effect of invasive species such as zebra mussels. As their populations increase in Lake Simcoe, they consume more algae thereby reducing the turbidity of the water resulting in sunlight reaching deeper areas (LSRCA, 2023).

A final component of the watershed health report card was the surface water quality. This was measured by the concentration of phosphorus, the health of the benthic invertebrates, and

chloride concentrations. The overall grading varied by location, with most of the watershed rating between poor and fair. Of the 15 samples taken for chloride evaluation recently, six were measured to be above the Canadian water quality guideline for long term exposure, and nine were found to be under the guideline (Figure 2.1) (LSRCA, 2023).

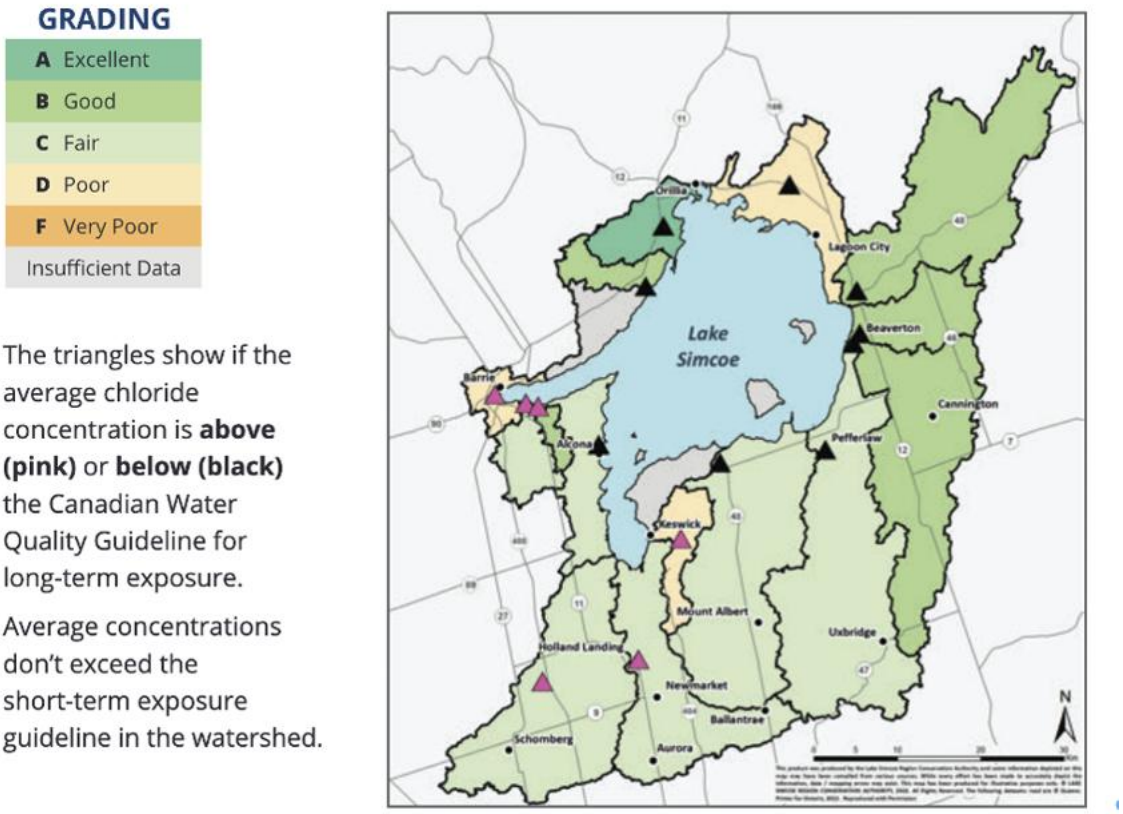


Figure 2.1- Surface Water Quality Report for the Lake Simcoe Watershed (LSRCA 2023)

The wetlands chosen for this research were selected based on their varying exposures to anthropogenic stressors, the ability to safely access the sampling points, and the presence of specific macrophytes *Typha angustifolia* (TA) and *Nymphaea odorata (*NO). Three of the four wetlands: Holland Marsh (HM) (Sierra Club, 2021), Lagoon City (LC) (LSRCA, 2023), and Victoria Point (VP) (Jaanusson, 2016), are all considered provincially significant wetlands.

Although Langman's Marsh (LM) has not been assessed by the Government, it is a conservation area privately owned and protected by the Orillia Fish and Game Conservation Club. The majority of the sites (three out of four) are fringe wetlands (Figure 2.2) (Lugo, 1990).

Figure 2.2 - A map of the Lake Simcoe Watershed (LSRCA, 2020).

2.2 Wetland Locations

2.2.1 Site 1: Langman's Marsh (LM)

The George Langman's Sanctuary is a 61 acre plot located at the southwest corner of Bass Lake Sideroad and the 14th Line of the township of Oro Medonte (GPS Coordinates: 44.6169, 79.5002; Figure 2.3). This marsh was dredged to create several canals to increase waterfowl activity. Water comes into Langman's via springs, and drains into Bass Lake

(OFGCC, 2023). The average depth of this Marsh is unknown, but through observations, the deepest area is thought to be approximately 2-3 meters, with an average depth varying from 1-2 meters depending on the section of the canals. The water is clear with a large amount of decomposing organic matter on the bottom. It is owned and maintained by the Orillia Fish and Game Conservation Club. Langman's is home to a considerable number of waterfowl, most commonly ducks, geese, and swans. The aquatic life includes small fishes, frogs, toads, water snakes, snails, and much more (Figure 2.4). Some of the major macrophytes found at this location were *Typha x glauca, Typha angustifolia, Typha latifolia, Nymphaea odorata, Rhus radicans, and Solidago canadensis* (Newmaster, 1997).

Figure 2.3 - Aerial view of Langman Marsh (OFGCC).

Figure 2.4 - Ground level view of Langman Marsh from the parking lot.

2.2.2 Site 2: Lagoon City (LC)

This site is a fringe wetland located on the northwest side of Lake Simcoe (GPS Coordinates: 44.56807, 79.2159; Figure 2.5). Lagoon City has many canals and lagoons and is located just under 2 kms from a sewage treatment plant that directly releases grey water into the lake (Jaanusson, 2016). The water is clear with a sandy bottom, and the average water depth along the marsh is approximately 1-1.5 meters. The major plants found at the sampling area were *Typha angustifolia, Sparganium eurycarpum, Nymphaea odorata, Typha x glauca, Scirpus acutus, and Brachythecium salebrosum* (Newmaster, 1997) (Figure 2.6).

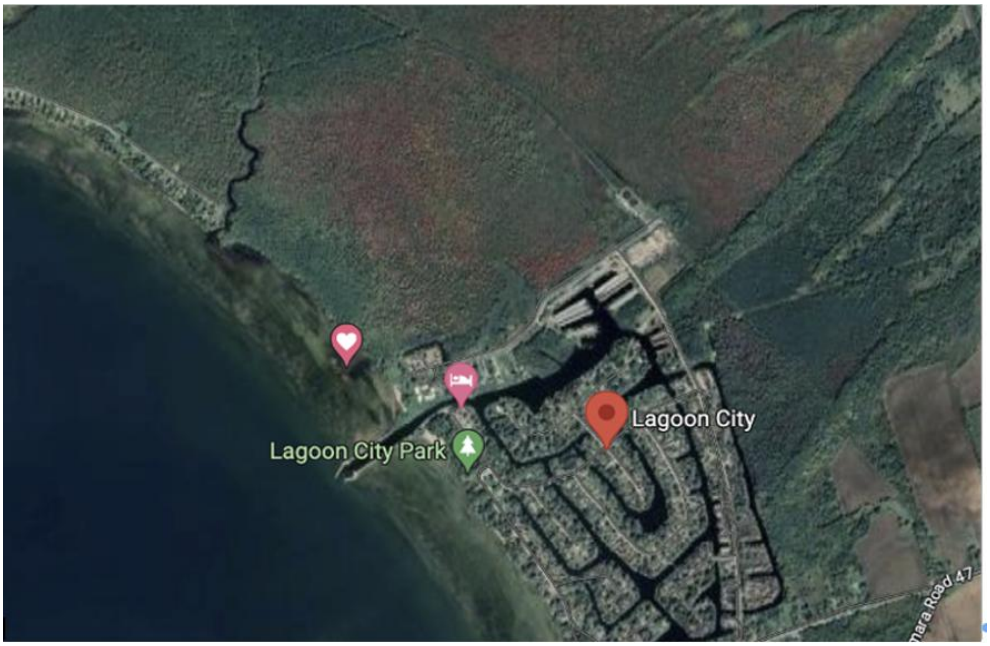


Figure 2.5 - Lagoon City Sampling point indicated with a pink heart.

Figure 2.6 - Lagoon City Coastal Wetland and Lake Simcoe view from a small boat launch.

2.2.3 Site 3: Holland Marsh (HM)

The Holland Marsh is a fringe wetland located south of Lake Simcoe (GPS Coordinates: 44.0946, 79.3115; Figure 2.7). The Holland Marsh wetland complex is the largest wetland habitat in the Lake Simcoe Watershed, with an area of 2,835 ha (Figure 2.8) (LSRCA). The Holland Marsh area is home to one of the most productive horticultural areas in Canada producing approximately 450 million dollars worth of crops and fulfilling a significant quantity of the vegetable demand in Ontario. The average depth of the entire marsh ranges from 2.5-3 meters (Madramootoo, 2022) while the average depth near the sampling location varied between 1-2 meters. The water is usually clear with low turbidity but contains a large amount of decomposing organic matter at the bottom. Holland Marsh is home to many different species of plants; some common ones seen around the sampling sites were *Nymphaea odorata, Typha angustifolia, Scirpus acutus, Typha xGlauca, Typha latifolia, Phragmites australis, Rhus radicans L., Spirodela polyrhiza, Lemna minor, and Calamagrotis canadensis* (Newmaster, 1997). Holland Marsh is exposed to several anthropogenic stressors such as boaters, large amounts of litter and fertilizers, and effluent from a wastewater treatment plant. A major problem is the runoff of fertilizers from local farms into the nearby canals which feed into the Holland Marsh thereby increasing the risk of eutrophication (DMAF, 2021). The Holland Marsh is considered a point of interest and a significant detriment to Lake Simcoe's health as it drains into the Holland River, which then drains directly into Lake Simcoe through Cooks Bay (Madramootoo, 2022).

Figure 2.7 - A visual of the Holland Marsh wetland sampling location indicated with a red pin drop, showing views of multiple farms in the area.

Figure 2.8 - A view of a small section of Holland Marsh wetland from a parking lot.

2.2.4 Site 4: Victoria Point (VP)

Victoria Point is a fringe wetland located on the northern end of Lake Simcoe, south of the connecting point between Lake Simcoe and Lake Couchiching (GPS Coordinates: 44.6111, 79.3876; Figure 2.9). The water is very turbid, with a brown and reddish colour (Figure 2.10) (Jaanusson, 2016). Victoria Point only receives water from Lake Simcoe (Jaanusson, 2016). The average depth of the sampling location varies between 1-2 meters. The site is full of toads, frogs, and birds, in the summer, along with some sightings of turtles. The sampling location is densely packed by *Typha angustifolia, Typha x glauca, and Typha latifolia. Nymphaea odorata, Sparganium eurycarpum, Lemna minor, Spirodela polyrhiza,* and *Brachythecium populeum*. Also seen are small numbers of *Scirpus acutus* and *Rhus radicans L* (Newmaster, 1997).

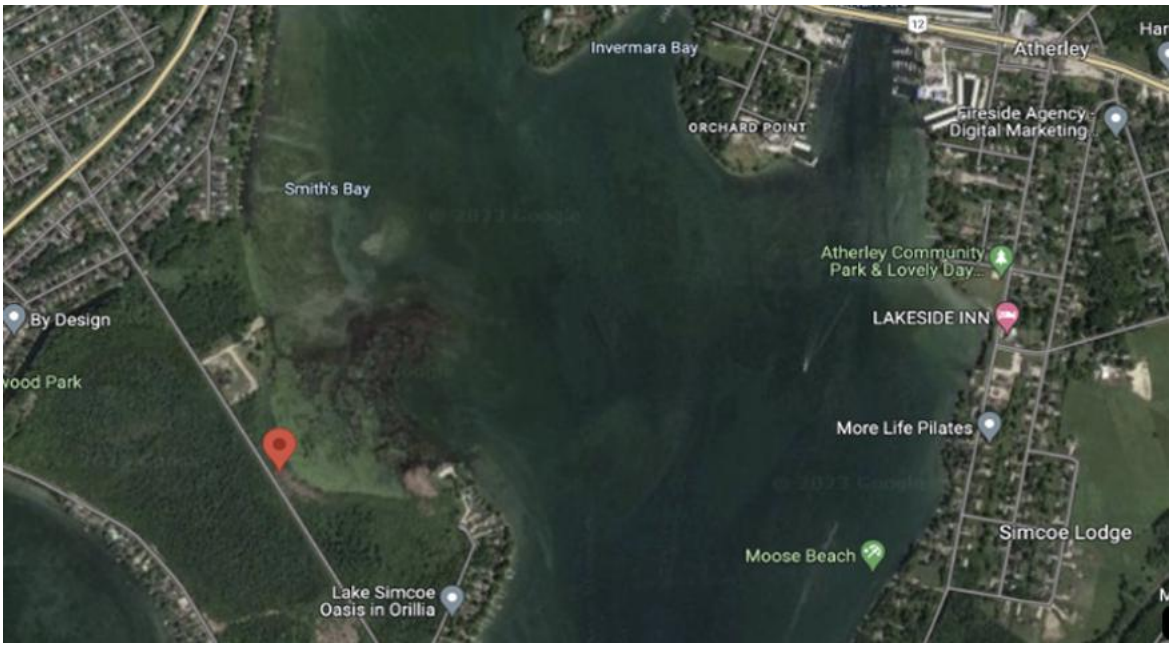


Figure 2.9 - The Victoria Point sampling location is indicated with a red drop point.

Figure 2.10 - Photo showing the densely packed Victoria Point wetland, with very turbid water, from a road view.

2.3 Macrophytes

The following two macrophyte species were selected for the study. This was because (1) these species represent two different types of macrophytes: the *Nymphaea odorata* (NO) are rooted and floating, and the *Typha angustifolia* (TA) are emergent, and (2) they were present in all four wetlands and in all the sampling intervals (seasons) of the study. These are some of the major macrophyte species found in this area.

2.3.1 *Typha angustifolia* alive (TA) and dead (TAD)

The plant species TA is from the cattail family known as Typhaceae. It is more commonly known as the narrow leaved cattail (Figure 2.11). It grows up to 6 ft tall. Their leaves

are 2-5 feet long, and are flattened, and narrower than the leaves of *Typha latifolia*. Their flowers are brown in colour and the cylindrical spike is velvety to the touch (USDA, 2006).

Typha angustifolia is native to Northern Africa, temperate Asia, and Eurasia. It is considered as an invasive species in North America and is especially of concern due to its high seed dispersion and thick monotypic groups (USGS, 2021). It can be found in wetlands, coastal areas, wet ditches, and fresh and brackish waters. They prefer wet, muddy, and full sun locations. They can thrive in locations of increased environmental stressors such as anthropogenic activities, continuous or seasonal water drawdowns, etc. *T. angustifolia* prefers shallower waters but can be seen in up to 2-3 meters deep waters (USGS, 2022).

The dead plant samples (TAD / *Typha angustifolia* dead) that were used in this research were typical TA plants that were only considered different from the "*Typha angustifolia* alive" or TA plants based on how old they were and the duration which they had been in the water. For example, TA plants were new this season and considered alive at the time of collection, while the TAD plants were much older, by at least one entire growing season, usually broken off or bent over into the water. The dead plants tend to be brown or yellow, soggy, and breaking apart, while the live samples tend to be much more firm, green, and standing upright (Figure 2.12). These different versions of the same macrophyte were collected to help understand the variation of epiphytes between the dead and live samples.

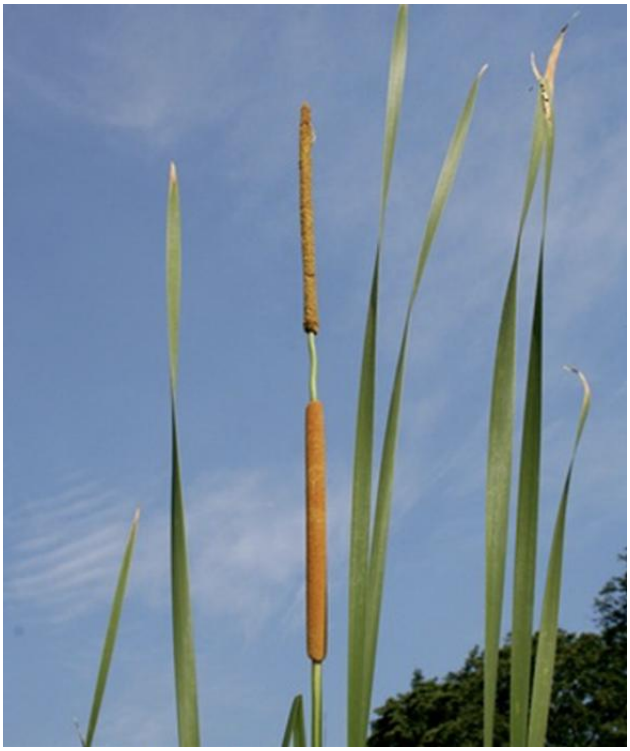


Figure 2.11- A photo of Typha angustifolia displaying the female/pistillate (bottom) and male/staminate (top) flowers, as well as the gap between them that is useful for identification. (Photo obtained from National Parks, 2023).

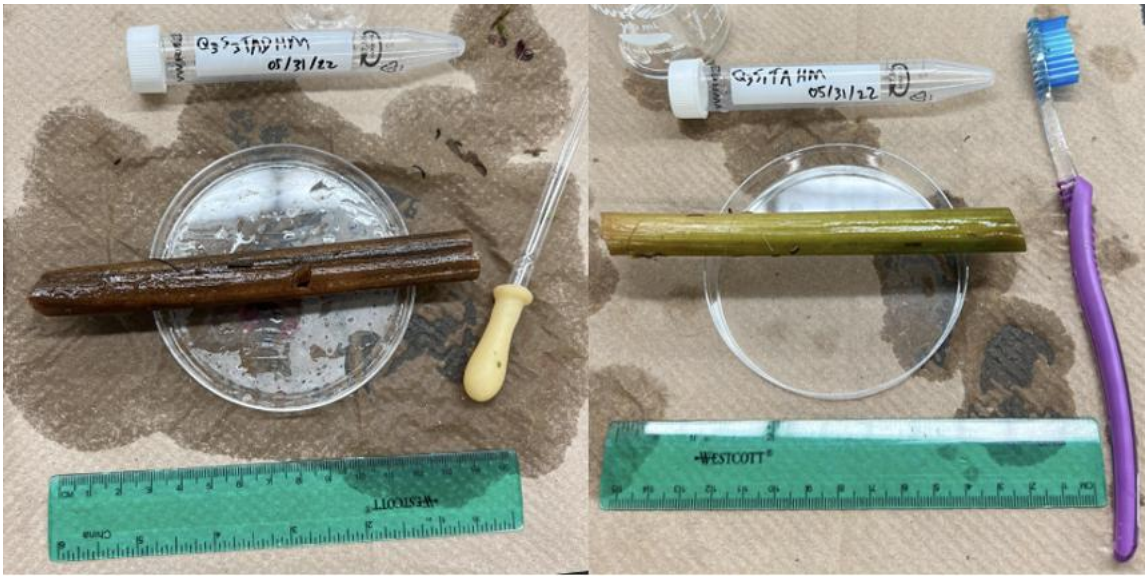


Figure 2.12 - A visual example of a TAD sample (Left) vs a TA sample (Right). Both samples are from Holland Marsh, collected and processed on the same day.

2.3.2 *Nymphaea odorata* (NO)

This macrophyte species belongs to the water lily family known as the Nymphaeaceae. It is more commonly known as a white-water lily and is native to most parts of North America (USDA, 2023). *N. odorata* is not considered native to Alaska, Hawaii, North Dakota, and Wyoming, and it is considered an invasive exotic species in California and Washington State (Invasive Plant Organization, 2018).

Nymphaea odorata is found in calm water up to 6 ft deep (USDA, 2023). It is a fast-spreading plant that thrives in freshwater lakes, ponds, and ditches or very slow-moving streams. It is a perennial macrophyte with large circular floating leaves attached to underwater stalks (Figure 2.13). The stalks rise from rhizomes attached to the ground, keeping the plant in place. The mature leaves measure around 25 cm across and have a greenish to reddish bottom. Their flowers are white with yellow centers and are attached to a single stalk that rises to the water surface to bloom. The flowers are on average 25 cm in diameter, they open in the day and close at night and are usually accompanied by 20 or more floating leaves. They are pollinated by beetles and bees and bloom for only up to approximately five days (USDA, 2023). After two to five days, the plant pulls the flower under the water surface, where the fruit breaks away (GISD, 2021). The fruit is a 2cm green capsule and contains oval seeds that are spread by the water flow after submersion which is the main way of reproduction (USDA, 2023; GISD, 2021). They can also do vegetative reproduction when parts of the rhizomes break off, float to a new location and start as a new colony there (USDA, 2023).

Figure 2.13 - An example of a Nymphaea odorata in the summer found at Langman's Marsh.

2.4 Sampling

2.4.1 Dates and Observations

Epiphytic algal sampling was conducted across four wetland locations, HM, VP, LC and LM during three seasonal periods (summer 2021, fall 2021, and spring 2022) to capture spatial and temporal variation in epiphyte community in different seasons (Table 2.1).

Table 2.1 - Sampling dates for each wetland in each season.

Season	Holland Marsh	Lagoon City	Langman's Marsh	Victoria Point
Spring	May 31st 2022	June 4th 2022	June 4th 2022	June 4th 2022
Summer	June 30th 2021	July 16th 2021	July 6th 2021	July 6th 2021
Fall	October 24th 2021	October 26th 2021	October 24th 2021	October 26th 2021

2.4.2 Sample Collection Methods

Triplicate samples of macrophytes (TA, TAD and NO) from different plants were collected from all the wetlands with care not to disturb the epiphytes. For each NO, submerged stem pieces were cut free from the plant and placed into a small, labelled ziploc bag. For each TA, the submerged portion of the stalks, approximately 5 inches long, were cut with cleaned scissors. Each sample was put in a labelled ziploc bag with water collected from the location to

keep the epiphytes submerged. Upon arrival in the lab, all macrophyte samples were stored in the refrigerator until they could be processed and analyzed (usually within the same day). Water samples were collected for phytoplankton and chlorophyll-*a* analyses from each wetland. The water samples for chlorophyll-*a* measurements were immediately processed. While the samples for the phytoplankton were stored in the refrigerator until ready for processing and analysis (within a day or two). Other water parameters such as dissolved oxygen (DO) (HQ40d multi probe), pH (sympHony SP70P probe), and conductivity (sympHony VWR probe) were all measured using the appropriate standardized probes directly at the sites.

2.4.3 Epiphyte Removal

Before extracting the epiphytes, the macrophytes were measured with the help of ruler. The NO leaves were measured from the top to bottom and from side to side. For TA and TAD, the length and width of the stems were measured with a ruler. All measurements were recorded, and photos of each plant sample were taken before the removal of epiphytes.

For the NO samples, the epiphytes were removed from a known area (5 x 6 cm) in the middle of the leaves. This was done by gently scrapping the bottom side of the leaves (the side touching the water) with the help of a clean toothbrush. The area was scrapped three times repeatedly with the help of 2 ml deionized water (DI water). Thus, a total of 6 ml sample was collected for each sample.

As for the TA (both live and dead samples), 4 x 2 cm area from the end of the stalk where the TA was cut and removed from the plant was scrapped with the help of a clean toothbrush. Here too, 2 ml of DI was used for scraping, and the process was repeated two more times to get a total of 6 ml of the epiphyte sample from each *T. augstifolia* sample.

The epiphyte samples were labelled correctly and stored in a 15ml centrifuge tube. To condense the sample, these tubes were centrifuged (Beckman Coulter Allegra X 22R Centrifuge) at 3000 rpm for 10 minutes. Three millilitre supernatant was removed, thus leaving 3 ml in each vial. The supernatant was observed under a microscope for any epiphytes before discarding. All epiphyte solutions were immediately stored in a refrigerator until they were analyzed under a compound microscope (Nikon Eclipse Si, 10x,20x,40x or 100x).

The scraping method was standardized by preliminary experiments to test the effectiveness of removing the epiphytes from the macrophytes and cleaning the brushes between samples. This was completed by repeated scraping on test sample epiphytes, rinsing, and viewing rinsed solution and the surface under a microscope (Nikon Eclipse Si,). Rinsing the brushes first, then soaking them for 2 minutes, then rinsing them again with tap water and then DI water, was the most successful way to ensure no cross contamination between samples. All sample analysis is completed within week or two after each sampling. All triplicate samples were analysed, and an average was taken for the data analysis.

2.4.4 Epiphyte Identification

The epiphyte samples were observed in a hemocytometer (Alwi, 2015) under a compound microscope (Nikon Eclipse Si, 10x,20x,40x or 100x) for species count and identification. The samples were identified using various identification keys and manuals (Wehr et al., 2015, Bellinger, Sigee, 2015). From the haemocytometer counts (average of eight counts for each sample) the density of the epiphytes was calculated and expressed as No./cm2 area of the macrophyte.

2.4.5 Phytoplankton analysis

The water samples for phytoplankton analysis were processed by condensing the water by centrifuging 1 litre of water sample down to 10 ml. The samples were centrifuged at 4000 rpm for 15 mins, and the supernatant was removed using a Pasteur pipette until the volume was reduced to the final 10 ml. The samples were observed using a haemocytometer (average of eight counts per sample) under a compound microscope (Nikon Eclipse Si, 10x,20x,40x or 100x) to identify the species and count them. The density of phytoplankton was expressed as No./L.

2.4.6 Water Parameter Measurements

The water parameters (DO, pH, conductivity, and temperature) were measured with the help of portable probes. Three 250 ml cleaned water bottles were used to collect water from each sampling location for nutrient analysis, total phosphorus, DOC, total nitrogen, and select contaminants such as aluminum, cadmium, zinc, etc. The bottled samples were labelled and stored in the freezer until analysis. The samples were sent to the Lakehead University Analytical Laboratory in Thunder Bay for nutrient analysis. Methods used by the Thunder Bay lab for the analysis can be found through the reference method codes in Table 2.2, metal analysis was completed but not used in this study.

Table 2.2 The reference method codes for the analysis methods and regulatory documents followed during the water analysis at Lakehead University in Thunder Bay.

PARCODE	PAR DESCRIPTION	MDL	UNITS	Method Code	Reference Method
WDOC	Dissolved Organic Carbon	0.5	mg/L	WDODIC	SKALAR 1102111
WTOTN	Total Nitrogen	0.015	mg/L	WTOTN	SKALAR 475-426
WTOTP	Total Phosphorous	0.005	mg/L	WTOTP	SKALAR 503-505
WICP1xx	Total Metals [Full scan]	----	mg/L	WICP1	EPA 3050

2.5. Chlorophyll- *a* analysis

For the chlorophyll-*a* measurement, one litre of water was filtered through 0.45 micron Whatman glass fibre filter paper. The chlorophyll was extracted in 12 ml of 90% acetone for 16-18 hours in a refrigerator. The acetone extract was centrifuged at 4000 rpm for 15 min under a low temperature, and the supernatant was extracted for optical density measurement in a UV visible spectrophotometer (Beckman Coulter DU730). The chlorophyll concentrations were calculated from the optical density readings. This analytical procedure was adapted from Strickland and Parsons (1968) and APHA (1992).

The chlorophyll-*a* concentrations were calculated by inserting the corrected optical density readings into the following equation ((11.85*(E_{664} E_{750}) 1.54*(E_{647} E_{750}) 0.08(E_{630} E_{750}))*V_e/L*V_f). Where: L= Cuvette light path in centimetres, V_e=Extraction volume in millilitres, V_f= filtered volume in a litre, and concentrations are in unit mg m^3 (Aminot, Rey 2000).

2.6 Calculations and data preparation

Several statistical analyses were conducted to get a deeper understanding of the community structure of epiphytes, their dynamics with season and wetlands, the importance of macrophyte hosts and the phytoplankton community in the Lake Simcoe's coastal wetlands (specific analysis conducted are explained in chapter 3, 4 and 5). The analyses were performed on the species richness, species diversity, total density, and per species density (PSD) of the epiphyte populations in relation to macrophytes, wetlands, seasons, and the phytoplankton genera. In addition, analyses were also done to study the influence of the water quality parameters on the epiphyte communities (Figure 2.14). By doing this, it is expected that we will have a deeper understanding of the use of epiphytes as bioindicators of wetland health, in

addition to the epiphyte's community variations with different macrophyte hosts found in these sampling locations.

Species diversity and species richness measurements: The Shannon Diversity Index calculation was used to calculate epiphyte and phytoplankton diversity in the samples (H = -SUM (Pi * ln(Pi))). Species richness is the total number of species observed in each sample.

Data transformation and homogeneity testing: Before running the statistical analysis, the data were subjected to log or square-root transformation. The species richness, density, and diversity were log transformed for both epiphyte and phytoplankton calculations. To help with the homogeneity of the data, the chlorophyll *a* concentration was square root transformed, while the conductivity readings and the DOC measurements were log transformed before the analysis. The dissolved oxygen, pH, water temperature, total nitrogen and total phosphorus data did not need any transformation.

The diagnostic plots were used to check the data and homogeneity of variance. The Anderson Darling test was used to check for normal distribution of the data set (Dodge, 2008). Interaction plots were used to visually examine for interactions. There were minor indications of crossovers. Diagnostic plots were used to recheck the data visually. Variance inflation factors were used to find and resolve any multicollinearity and help to create the best models for the data analysis (Penn State, 2023).

2.7. Methods Flowchart

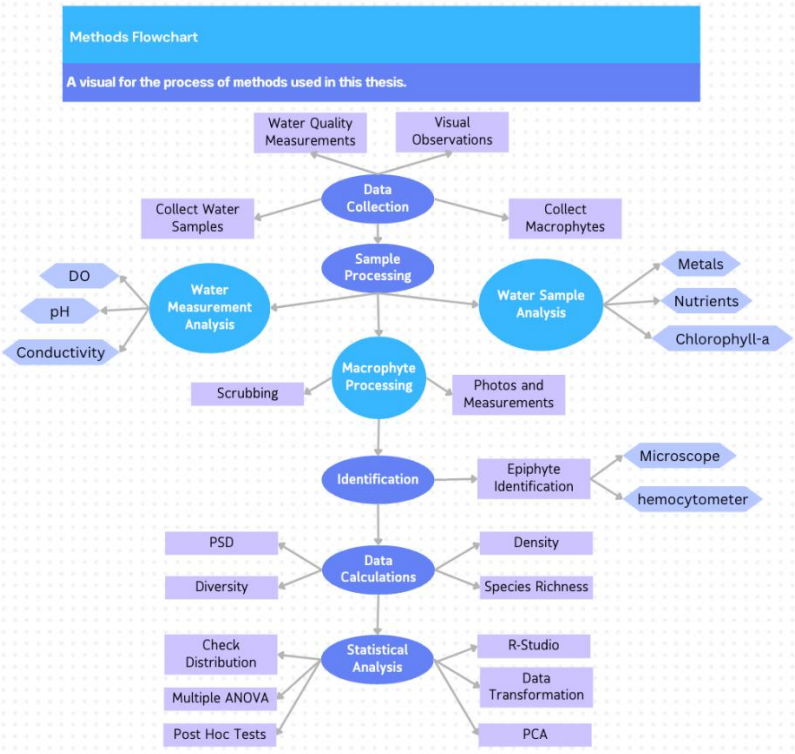


Figure 2.14 - Methods process flowchart from measurements and sampling to statistical analysis.

Chapter 3 : The Variation of Environmental Parameters in the Wetlands

3.1 Introduction

Water quality is a measurement of biological, microbial, chemical, and physical characteristics of water. Testing water quality provides empirical evidence supporting the current or emerging environmental impacts on aquatic life and the surrounding ecosystem (USEPA, 2023). The water quality parameters measured in this study were pH, water temperature, conductivity, total phosphorus (TP), total nitrogen (TN), dissolved organic carbon (DOC), dissolved oxygen (DO), chlorophyll-*a* concentration and the total metal content. These parameters were evaluated in spring, summer, and fall at each wetland site.

The optimal pH of lake water is essential for plant and animal life, outside the normal range, several species do not survive. Dissolved Oxygen (DO) is another fundamental water quality indicator vital for most aquatic life. The DO sources include atmospheric oxygen mixing and photosynthesis of aquatic plants. Insufficient DO threatens the diversity and survival of aquatic organisms (Zhang, 2023; CCME, 1999).

Water temperature has an important impact on aquatic ecosystems. In lentic freshwater systems such as lakes, thermal stratification poses a major issue for aquatic organisms. Thermal stratification is a natural occurrence in aquatic ecosystems where water forms into distinct layers based on temperature and density variations. The stratification results in three main layers: the warm epilimnion at the surface, the quickly changing metalimnion in the middle, and the cooler, dense hypolimnion at the bottom. Atmospheric temperature fluctuations influence these layers. Therefore, the stratification becomes more pronounced during the warmer summer season (USGS, 2019). As the temperature differences between these layers increase, the deeper layers can experience a depletion in dissolved oxygen, negatively impacting the aquatic life in those

areas (USGS, 2022). While thermal stratification is a natural phenomenon, a sustained elevated temperature can synergize its effects by potentially degrading the water quality (Woolway et al., 2020).

While the total phosphorus and nitrogen support a healthy food web, significant increases in these nutrients support rapid algal growth altering the balance and health of the ecosystem (EPA, 2012). Aquatic ecosystems require a nutrient balance. An overly nutrient rich body of water is characterized by abundant aquatic plants, excess phytoplankton growth, reduced oxygen, increased conductivity, etc. Conversely, an oligotrophic lake has minimal nutrient concentration and limited aquatic life. A mesotrophic lake sits between these two, with moderate nutrient and plankton growth (NHDES, 2019).

Dissolved organic carbon, if in excess, can disrupt the ecosystem's chemistry, affect pH levels, and impede light penetration (Lawrence et al., 2021). The chlorophyll-*a* correlates to algal biomass in bodies of water and indicate the primary productivity (EPA, 2023). The conductivity indicates the water's electric current transmission capacity which reflects its dissolved contaminant concentration levels (EPA, 2023).

To conclude, the changes in water parameters with seasons in temperate lakes result in variations in growth rate, species diversity and abundance of aquatic organisms. This includes variations in microscopic phytoplankton and epiphyte communities. Understanding these environmental fluctuations and their relationships with microscopic algal communities is essential to understanding how these fluctuations shape epiphyte community structures in lentic water bodies and therefore use the epiphytes as indicators of water quality changes.

3.2 Methods

The description of methods of collection of water samples and water parameters was given in Chapter 2 (Materials and Methods). The results of the measurements and sample analyses are described below.

Statistical analysis:

To understand the complex overlap and variation in water parameters across wetlands, univariate and multivariate statistical analyses were performed. To test for significant differences among wetlands and seasons, multivariate analyses of variance (MANOVA) were applied to each water quality parameter pH, water temperature (Temp), dissolved oxygen (DO), conductivity (Cond), total nitrogen (TN), total phosphorus (TP), dissolved organic carbon (DOC), and chlorophyll-*a* (Chloro) (Quinn & Keough, 2002). This approach allowed for the identification of whether each parameter exhibited significant spatial heterogeneity between sites and temporal variability across sampling periods. MANOVAS were used because they provide a robust method to evaluate mean differences in continuous variables across multiple groups, while also being flexible enough to handle ecological datasets with moderate sample sizes. Following the MANOVAs, Tukey's Honest Significant Difference (HSD) post hoc tests were applied to determine which pairwise comparisons between wetlands or seasons accounted for the significant results (Sokal & Rohlf, 1995). The use of Tukey's HSD was particularly important in this context, as it reduces the risk of inflated Type I error associated with multiple comparisons, while still providing clear identification of specific site or seasonal contrasts. These analyses together enabled both a broad and fine scale understanding of how water quality parameters varied within and across the wetlands.

A Principal Component Analysis (PCA) was then conducted on a standardized dataset containing eight variables (pH, water temperature, dissolved oxygen, conductivity, total nitrogen, total phosphorus, dissolved organic carbon, and chlorophyll-*a*) to reduce dimensionality and identify the dominant environmental gradients (Legendre & Legendre, 2012). PCA was chosen because it provides a visual and statistical summary of correlated environmental factors, highlighting axes of maximum variance explained. All analyses were performed using R statistical software (R Core Team, 2024) within R-Studio (R-Studio Team, 2023).

3.3 Results

3.3.1 Water Quality

pH

During the study period, the pH in the wetlands varied between 7.59 - 8.34 in HM, 7.77 - 8.57 in LC, 7.12 - 7.55 in LM and 5.63 - 7.61 in VP, respectively. The mean readings of pH were 8.21$\pm$0.40 in LC, 7.81$\pm$0.46 in HM, 7.39$\pm$0.23 in LM, and 6.79$\pm$1.03 in VP. The mean seasonal values of pH were 7.83 (spring), 7.82 (summer) and 7.01 (fall), respectively. In HM and LM, the pH was highest in the spring and lowest in the fall. In contrast, LC and VP exhibited the peak pH values in the summer, followed by spring, with the lowest in the fall. VP demonstrated the greatest seasonal variability in pH, while LM showed the least variation. Among all sites, LC recorded the highest pH value overall, whereas VP recorded the lowest (Table 3.1).

The results of a one-way ANOVA revealed that pH levels across the wetlands were significantly different ($p<0.005$). A Tukey HSD test indicated that the pH readings between all tested wetlands were significantly different from each other ($p<0.05$). A one-way ANOVA showed significant differences in pH among the seasons ($p<0.001$). A Tukey HSD showed the seasonal pH levels were significantly different, specifically between fall and spring, as well as

between fall and summer ($p<0.005$), while no significant difference was found between spring and summer ($p>0.05$).

Table 3.1 - Variation of pH with standard deviation (SD) at the sampling locations over the three seasons.

Wetland	Spring	Summer	Fall	Average	Standard Deviation
Holland Marsh	8.34	7.59	7.5	7.81	0.46
Lagoon City	8.29	8.57	7.77	8.21	0.40
Langman's Marsh	7.55	7.51	7.12	7.39	0.23
Victoria Point	7.14	7.61	5.63	6.79	1.03

Water Temperature

The water temperature in the wetlands during the study period varied between 12.80-27.30℃ in HM, 8.90 - 31.30℃ in LC, 7.70 - 23.30℃ in LM, and 10.80 - 22.70℃ in VP. The mean readings of water temperature were 20.33℃ $\pm$ 7.27 in HM, 19.00℃ $\pm$ 11.36 in LC, 16.93℃ $\pm$ 5.96 in VP, and 16.63℃ $\pm$ 8.04 in LM. The mean seasonal values of water temperature were 23.45℃ (Summer), 21.8℃ (Spring), and 10.05℃ (Fall), respectively. In HM and LM, the highest water temperatures were recorded in the spring, with the lowest in the fall. Conversely, LC and VP experienced peak temperatures in the summer and the lowest in the fall. HM recorded the highest overall temperature among all sites, while LM had the lowest. VP exhibited the least seasonal variation in water temperature relative to the other wetlands (Table 3.2).

The results of a one-way ANOVA revealed that water temperatures across the selected wetlands were not significantly different ($p>0.05$). The one-way ANOVA results for water temperature indicated significant differences between seasons ($p<0.005$). Subsequently, Tukey HSD analysis confirmed the distinct variations across all seasons for each variable.

Table 3.2 Variation of water temperature with standard deviation (SD) between the sampling locations.

Wetland	Spring	Summer	Fall	Average	Standard Deviation
Holland Marsh	27.3	20.9	12.8	20.33	7.27
Lagoon City	16.8	31.3	8.9	19.00	11.36
Langman's Marsh	23.3	18.9	7.7	16.63	8.04
Victoria Point	17.3	22.7	10.8	16.93	5.96

Dissolved Oxygen

During the study period, the DO range in the wetlands varied between 1.29 - 9.76 mg/L in HM, 9.06 - 12.71 mg/L in LC, 4.23 - 7.96 mg/L in LM, and 2.47 - 8.61 mg/L in VP. Mean readings of DO between wetlands were 11.08 $\pm$ 1.86 mg/L in LC, 6.37 $\pm$ 1.92 mg/L in LM, 6.07 $\pm$ 4.34 mg/L in HM, and 5.23 $\pm$ 3.12 mg/L in VP. The mean seasonal values of DO were 7.80 mg/L (Fall) , 7.05 mg/L (Spring), and 6.71 mg/L (Summer), respectively. LC and VP recorded their lowest DO levels in the spring and highest in the summer. In contrast, HM exhibited its highest DO concentrations in the spring and lowest in the summer. LM peaked DO levels in the fall and lowest in the summer. Overall, LC had the highest dissolved oxygen concentrations across all seasons. The greatest seasonal variation in DO was observed in HM, while LC exhibited the most consistent DO levels throughout the year (Table 3.3).

Dissolved oxygen measurements differed significantly between wetlands ($p<0.005$). The Tukey HSD test identified LC's DO levels differed from all other sampled wetlands. DO concentrations remained consistent across all seasons and the one-way ANOVA results showed no significant seasonal differences ($p>0.05$).

Table 3.3 - Variation of dissolved oxygen with standard deviation (SD) between the sampling locations.

Wetland	Spring	Summer	Fall	Average	Standard Deviation
Holland Marsh	9.76	1.29	7.15	6.07	4.34
Lagoon City	9.06	12.71	11.48	11.08	1.86
Langman's Marsh	6.91	4.23	7.96	6.37	1.92
Victoria Point	2.47	8.61	4.6	5.23	3.12

Conductivity

During the study period, conductivity values (in µS/cm) varied widely across wetlands and seasons. HM exhibited the highest conductivity overall, ranging from 665.00 µS/cm in spring to 913.00 µS/cm in fall. LM ranged from 401.00 µS/cm in spring to 444.00 µS/cm in summer, while LC ranged from 476.00 µS/cm in summer to 506.00 µS/cm in spring. VP had the lowest values overall, ranging from 213.00 µS/cm in fall to 541.00 µS/cm in summer. The highest conductivity was observed at HM in the fall, and the lowest at VP in the fall. The average conductivity by wetland was 782.00 µS/cm (±124.59) for HM, 490.67 µS/cm (±15.01) for LC, 423.67 µS/cm (±21.59) for LM, and 350.13 µS/cm (±170.47) for VP (Table 3.4). Seasonal averages were 510.50 µS/cm in the fall, 517.75 µS/cm in the spring, and 557.25 µS/cm in the summer. These results show that HM consistently exhibited elevated conductivity, likely indicating higher ion concentrations, while VP had the lowest mean conductivity and the highest variability. Seasonal variability was modest overall, with slightly higher conductivity in summer across most sites.

The one-way ANOVA revealed significant differences in conductivity among all wetlands ($p<0.05$). However, the Tukey HSD test found that all wetland comparisons significantly differed except for LM and LC ($p>0.05$). Results for conductivity indicated

significant differences between seasons ($p<0.05$). Subsequently, a Tukey HSD analysis showed that a significant difference was detected between summer and spring ($p<0.05$).

Table 3.4 - Variation of the conductivity with standard deviation (SD) between the sampling locations.

Wetland	Spring	Summer	Fall	Average	Standard Deviation
Holland Marsh	665.0	768.0	913.0	782.0	124.59
Lagoon City	506.0	476.0	490.0	490.67	15.01
Langman's Marsh	401.0	444.0	426.0	423.67	21.59
Victoria Point	296.40	541.0	213.0	350.13	170.47

Total Nitrogen

The Total Nitrogen (TN) range in the wetlands during the study period varied between 0.69- 1.05 mg/L in HM, 0.37 - 0.48 mg/L in LC, 0.67- 1.79 mg/L in LM, and 0.58 - 1.90 mg/L in VP. The rankings of Total Nitrogen between wetlands were 1.14 $\pm$ 0.68 mg/L in VP, 1.07 $\pm$ 0.62 mg/L in LM, 0.89 $\pm$ 0.19 mg/L in HM, and 0.42 $\pm$ 0.06 mg/L in LC. The mean seasonal values of Total Nitrogen were: 1.05 mg/L in the fall, 0.92 mg/L in the summer, and 0.68 mg/L in the spring, respectively. In HM, the highest TN concentration was recorded in the fall and the lowest in the spring. Both LC and VP also exhibited peak TN levels in the fall and the lowest in the summer. In contrast, LM recorded its highest TN in the summer and lowest in the spring. LC showed the least seasonal variation in TN, while VP exhibited the greatest seasonal fluctuation and recorded the highest average TN concentration among all wetlands (Table 3.5).

The one-way ANOVA revealed significant differences in the TN concentrations between wetlands ($p<0.005$), and the post hoc analyses using the Tukey HSD test identified that only LC displayed significant difference in TN when compared to all other sites ($p<0.005$).

Seasonal variations in TN were markedly pronounced for LM and VP. A one-way ANOVA showed a significant difference for TN between the seasons tested ($p<0.005$). The Tukey HSD test showed that the total nitrogen significantly differed between spring and fall ($p<0.05$).

Table 3.5 - Variation of total nitrogen with standard deviation (SD) between the sampling locations

Wetland	Spring	Summer	Fall	Average	Standard Deviation
Holland Marsh	0.69	0.94	1.05	0.89	0.19
Lagoon City	0.41	0.37	0.48	0.42	0.06
Langman's Marsh	0.67	1.79	0.75	1.07	0.62
Victoria Point	0.93	0.58	1.9	1.14	0.68

Total Phosphorous

During the study period, TP concentrations (mg/L) varied among the wetlands. Observed ranges were: 0.02 - 0.07 mg/L in HM, 0.01 - 0.07 mg/L in LC, 0.01 - 0.04 mg/L in LM, and 0.01 -0.08 mg/L in VP. When comparing mean TP concentrations between wetlands, the ranking was: 0.05 ± 0.03 mg/L in HM, 0.04 ± 0.04 mg/L in VP, 0.03 mg/L $\pm$ 0.02 in LM, and 0.03 ± 0.03 mg/L in LC, respectively. The mean seasonal values of TP concentrations were highest in spring (0.06 mg/L), followed by summer (0.04 mg/L), and lowest in the fall (0.02 mg/L) (Table 3.6). These results highlight both spatial and temporal variability in phosphorus levels, with elevated concentrations generally observed in the spring.

The one-way ANOVA results showed that the TP concentrations significantly differed between wetlands ($p<0.005$) and displayed temporal fluctuations. A Tukey HSD test identified that only LM and HM showed a significant difference in TP between each other ($p<0.005$). The One-way ANOVA results for TP indicated significant differences between seasons ($p<0.005$). A Tukey HSD analysis confirmed that TP varied between each pair of seasons

Table 3.6 - Variation of total phosphorous with standard deviation (SD) between the sampling locations.

Wetland	Spring	Summer	Fall	Average	Standard Deviation
Holland Marsh	0.06	0.07	0.02	0.05	0.03
Lagoon City	0.07	0.01	0.02	0.03	0.03
Langman's Marsh	0.01	0.04	0.04	0.03	0.02
Victoria Point	0.08	0.03	0.01	0.04	0.04

Dissolved Organic Carbon

The DOC concentrations varied notably across wetlands and seasons during the study period. The observed ranges were 13.90 - 22.53 mg/L in HM, 4.13 - 6.77 mg/L in LC, 0.73 - 12.10 mg/L in LM, and 9.90 - 113.22 mg/L in VP. Mean rankings of the DOC concentrations were approximately 55.29 ± 3.39 mg/L in VP, 18.20 ± 1.30 mg/L in HM, 11.22 ± 0.78 mg/L in LM, and 5.50 ± 0.38 mg/L in LC (Table 3.7). Seasonal variations in DOC levels were evident across all sites. In HM and LM, DOC concentrations were lowest in the fall, whereas LC and VP exhibited their lowest values during the summer. The highest DOC concentration in HM occurred in the summer, while LC and VP had their DOC peak in the fall. LM was the only site where DOC reached its highest level in the spring; however, it also exhibited minimal variability, with a standard deviation equivalent to about ±0.78 mg/L on the linear scale. HM had the highest average DOC concentration at approximately 18.20 mg/L, while LC had the lowest at roughly 5.50 mg/L.

DOC significantly varied between wetlands. VP had pronounced seasonal variations in DOC. A one-way ANOVA found that the DOC levels significantly differed across all wetlands ($p<0.005$). A Tukey HSD test showed significant differences specifically between summer and fall.

Table 3.7 - Variation of dissolved organic carbon with standard deviation between the sampling locations.

Wetland	Spring	Summer	Fall	Average	Standard Deviation
HM	19.5	22.39	13.8	18.2	4.61
LC	5.89	4.17	6.76	5.50	1.39
LM	12.02	11.22	10.72	11.22	0.78
VP	42.66	10.0	113.22	55.29	43.08

Chlorophyll-*a*

The Chlorophyll-*a* concentration range per wetland was measured as HM: 0.883-1.1589(µg/L), LC: 0.90- 1.616(µg/L), LM: 1.148-1.395(µg/L), VP: 0.583-2.000(µg/L). The mean rankings of Chlorophyll-*a* between wetlands were: 1.38 $\pm$ 0.41 mgL^{1} in LC, 1.36 $\pm$ 0.72 mgL^{1} in VP, 1.23 $\pm$ 0.14 mgL^{1} in LM, and 1.04 $\pm$ 0.14 mgL^{1} in HM. The mean seasonal values of Chlorophyll-*a* between seasons were 1.48 mgL^{1} (Summer), 1.28 mgL^{1} (Spring), and 0.99 mgL^{1} (Fall), respectively. In HM, the lowest chlorophyll-*a* levels were observed in the spring, with the highest value in the summer. LC and VP recorded their highest chlorophyll-a concentrations in the summer and lowest in the fall. LM exhibited a different pattern, with peak values in the fall and the lowest in the spring. LC had the highest average chlorophyll-*a* concentration across all sites, while HM recorded the lowest average. LC also demonstrated the greatest seasonal variation in chlorophyll-*a* levels, whereas HM showed the least (Table 3.8).

One-way ANOVA showed the Chlorophyll-*a* levels varied significantly ($p<0.005$) among wetlands. The Tukey HSD test showed that only HM - LC and VP - HM were significantly different. The One-way ANOVA results for chlorophyll-*a* varied significantly across all wetlands ($p<0.005$). The Tukey HSD analysis confirmed that significant variations were detected between each pair of wetlands.

Table 3.8 - Variation of chlorophyll-a with standard deviation between the sampling locations.

Wetland	Spring	Summer	Fall	Average	Standard Deviation
Holland Marsh	0.88	1.16	1.07	1.04	0.14
Lagoon City	1.6	1.62	0.90	1.38	0.41
Langman's Marsh	1.15	1.15	1.40	1.23	0.14
Victoria Point	1.49	2.00	0.58	1.36	0.72

3.3.3 PCA Results

To further investigate the relationships among water-quality parameters across wetlands, a Principal Component Analysis (PCA) was conducted using eight standardized variables: water temperature, total phosphorus (TP), total nitrogen (TN), dissolved organic carbon (DOC), dissolved oxygen (DO), conductivity, pH, and chlorophyll-*a*. To reduce dataset dimensionality and identify the principal gradients explaining variation in wetland water quality. The first two components accounted for approximately 68% of the total variance, with PC1 (49%) represented on the y-axis and PC2 (18.8%) on the x-axis (Figure 3.1).

PC1 (y-axis, 49%) represented a dissolved oxygen versus phosphorus/productivity gradient, with strong positive loadings for DO and strong negative loadings for TP, along with contributions from water temperature, conductivity, and chlorophyll-*a*. Wetlands with higher PC1 scores exhibit more oxygenated conditions (e.g., LM, LC), whereas wetlands with lower PC1 scores (e.g., VP and HM) correspond to more nutrient rich, warmer, and higher productivity waters.

PC2 (x-axis, 18.8%) captured a nutrient and organic matter gradient, characterized by strong positive loadings for TN and DOC and negative loadings for pH and conductivity. Wetlands on further the right side of PC2 (e.g., VP) displayed elevated TN and DOC concentrations, reflecting organic and nutrient enrichment, whereas wetlands on the left side

(e.g., LC) exhibited lower nutrient and DOC levels, consistent with less impacted conditions (Figure 3.2).

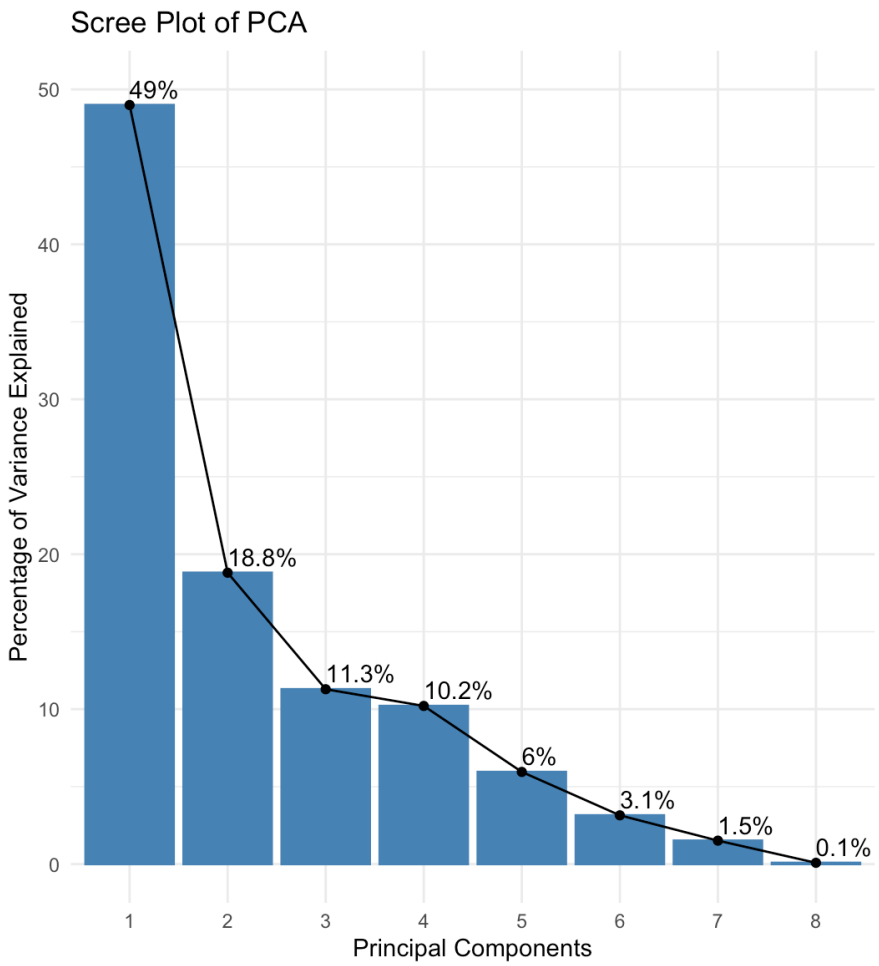


Figure 3.1 - Scree plot of principal component analysis (PCA) showing that PC1 explains 49% of the variance and PC2 explains 18.8%, together capturing 67.8% of the variability in the dataset.

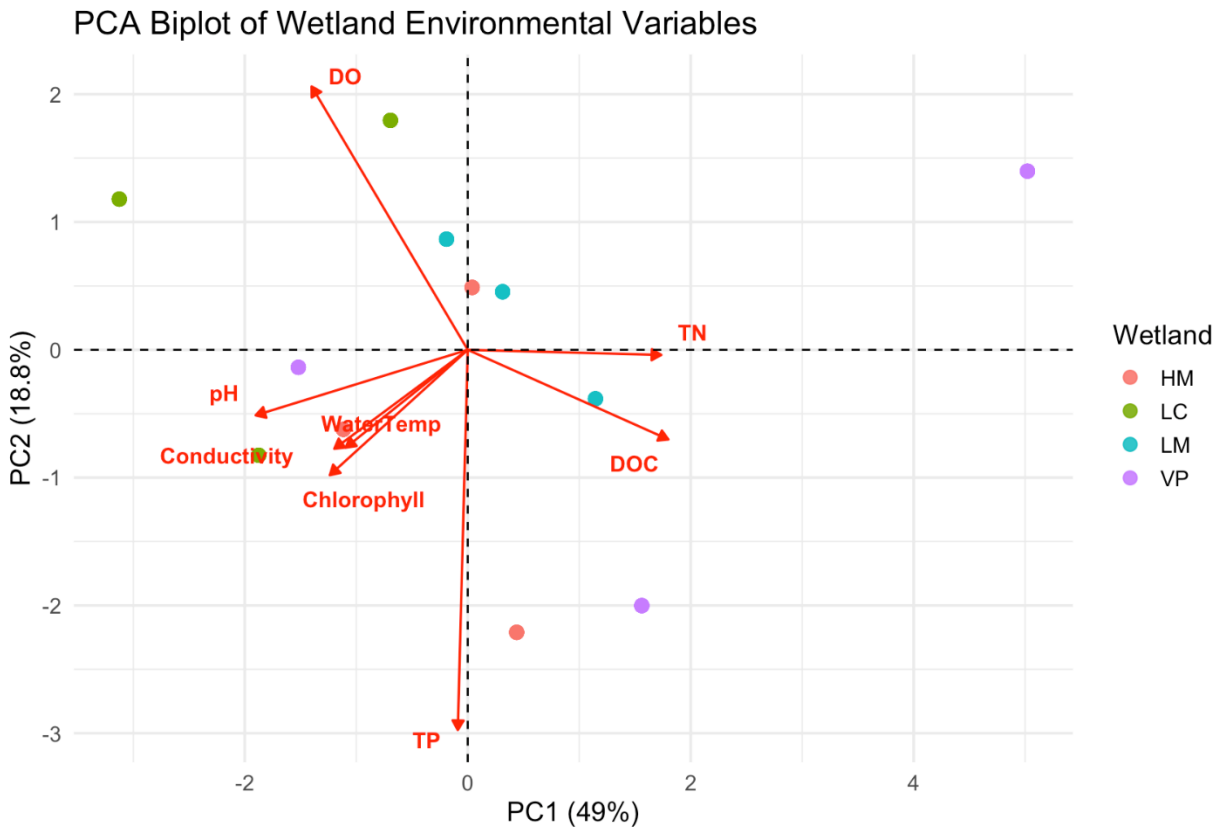


Figure 3.2 - Principal Component Analysis (PCA) biplot of wetland environmental variables. PC1 (49%) represents the primary oxygen-nutrient gradient. PC2 (18.8%) captures a secondary nutrient and organic matter gradient defined by TN and DOC. Wetland sites.

3.4 Discussion

3.4.1 Variations of Water Quality Parameters Between Wetlands

Water quality parameters in freshwater wetlands are influenced by a combination of natural processes and anthropogenic pressures. In this study, significant variation was observed in pH, TP, DOC, TN, DO, conductivity, and chlorophyll-*a* across the four sampled wetlands-VP, LM, HM, and LC, over three seasons (spring, summer, fall). These variations offer insight into the influence of surrounding land use, seasonality, nutrient enrichment, and the ecological effects of wetland chemistry.

pH

The pH exhibited significant variation across all wetlands and seasons, with notable spatial and temporal patterns. LC recorded the highest pH values, peaking at 8.57 in the summer. This is consistent with elevated photosynthetic activity from algae and aquatic macrophytes, which consume CO_2 and raise pH (U.S. EPA, 2023). The summer peak at LC coincided with high sunlight and warm temperatures conducive to algal growth. In contrast, VP registered the lowest pH value (5.63) in fall, likely due to increased organic inputs from decomposing vegetation and tannins (FOCA, 2021). Urban runoff containing acidic compounds may also have contributed, given the site's proximity to Orillia's wastewater facility and residential development (Environment Canada, 1996).

Langman's Marsh exhibited relatively neutral and stable pH values throughout the study period, suggesting limited anthropogenic input and a well buffered system. According to the Lake Simcoe Region Conservation Authority (LSRCA, 2022), pH stability in marshes like LM reflects minimal disturbance and a robust capacity to neutralize acidic or basic inputs. These patterns highlight the sensitivity of pH to both biotic activity and external stressors. Seasonally, the greatest pH decline occurred in the fall, supporting the role of organic decomposition and cooler temperatures in acidification (McIntyre et al., 2018). Wetlands across Ontario typically range between pH 6.0-8.0, with higher values in wetlands receiving agricultural runoff (Stantec Consulting Ltd., 2013).

Dissolved Oxygen

DO levels were significantly higher in LC than in other wetlands, possibly due to the increased wind mixing, hydrological inflow, or boating. Elevated DO enhances habitat suitability for aerobic organisms. In contrast, VP and HM occasionally recorded DO below 5 mg/L, a stress

threshold for aquatic life. These low levels likely result from microbial decomposition of DOC consuming oxygen (U.S. EPA, 2023). The inverse DO-DOC relationship was most evident in VP, suggesting organic enrichment drives oxygen depletion (Chapelle et al., 2012).

Seasonal DO variation was modest, but slightly higher in cooler spring and fall water consistent with temperature dependent oxygen solubility (Wetzel, 2001). Ontario wetlands often show summer DO declines due to decomposition and plant respiration (Snodgrass et al., 2008). In stagnant wetlands, DO can fall below critical thresholds at night or under ice (Crosbie & Chow-Fraser, 1999). Runoff driven nutrient inputs intensify this by stimulating microbial oxygen demand (Stewart & Kantrud, 2002). Seasonal DO results were non-significant.

Conductivity

Conductivity differed significantly among wetlands, with HM highest and VP lowest. Conductivity reflects dissolved ions such as nitrates, phosphates, and salts, commonly introduced via agricultural or urban runoff (Smith et al., 2020). HM's elevated conductivity aligns with its nutrient levels and proximity to farmland and wastewater sources. No significant seasonal effect was found, though the fall variability suggests episodic pollution pulses. LC and LM had similar moderate conductivity; LC's urban runoff and LM's road adjacency likely contribute. In southern Ontario, wetlands near developed areas often exceed 700 µS/cm (LSRCA, 2020; Environment Canada, 2001), matching HM's readings. Elevated conductivity can alter aquatic invertebrate and plant communities (Snodgrass et al., 2008).

Total Nitrogen

TN varied significantly among wetlands. LC had the lowest TN, likely due to efficient internal cycling and denitrification promoted by higher DO (Song et al., 2024). TN concentrations below approximately 0.5 mg/L characterize oligotrophic conditions, values

between 0.5-1.0 mg/L correspond to moderate, mesotrophic nutrient levels, and concentrations exceeding 1.0 mg/L indicate eutrophic conditions with heightened risk of elevated algal productivity (Liu et al., 2020). Across the wetlands studied, TN values spanned a wide range and intersected all three threshold categories. LC consistently measured from oligotrophic to mesotrophic conditions. HM and LM generally fell within the mesotrophic range, and VP measured from mesotrophic to eutrophic levels. Seasonal averages further supported these patterns, with TN increasing from spring (0.68 mg/L) to summer (0.92 mg/L) and peaking in fall (1.05 mg/L), indicating a shift toward eutrophic conditions later in the growing season. Together, these results suggest that nutrient availability - and therefore potential for algal production - varies substantially both among wetlands and across seasons within the region (Liu et al., 2020; Chambers et al., 2006).

Total Phosphorus

TP concentrations differed substantially among wetlands and seasons, aligning the wetlands with different trophic categories used in Canadian water-quality reporting. According to Environment and Climate Change Canadia (ECCC, 2023), the phosphorus concentration in meso-eutrophic conditions range between 0.020-0.035 mg/L, while eutrophic conditions range from 0.035 - 0.100 mg/L. Based on these thresholds, LC and LM fell within the meso-eutrophic range, with mean concentrations of approximately 0.02-0.03 mg/L, indicating moderate nutrient enrichment. In contrast, VP and HM were consistently eutrophic, with average concentrations between 0.04-0.08 mg/L, suggesting a higher nutrient loading (ECCC, 2023). VP displayed the widest TP range (0.01-0.08 mg/L), indicating episodic phosphorus inputs, likely associated with short-term runoff events or internal sediment release. HM and LC showed pronounced spring spikes, a pattern commonly linked to snowmelt driven fertilizer runoff (LSRCA, 2021). HM and

VP most frequently reached upper mesotrophic to eutrophic conditions, aligning with their elevated phosphorus concentrations. These elevated values suggest higher external loading. In contrast, LM and LC generally remained in the mesotrophic range, indicating a more moderate nutrient level and lower risk of eutrophication compared to VP and HM. This gradient reflects how watershed characteristics, land use, and hydrologic connectivity shape nutrient dynamics in small wetland systems (LSRCA, 2021).

Dissolved Organic Carbon

DOC varied significantly by wetland and season. VP recorded the highest DOC, consistent with its dense vegetation, reduced flow, and humic input from decomposing litter, explaining its dark color and low pH (Dadi et al., 2021). LC had the lowest DOC due to greater openness and constant flushing. Elevated DOC decreases light penetration and increases oxygen demand, contributing to observed DO depletion (Wetzel, 2001). VP recorded the highest DOC, and the lowest DO - similarly LC recorded the lowest DOC and the highest DO.

Chlorophyll-*a*

Chlorophyll-*a* varied across sites and seasons, tracking nutrient availability, temperature, and light availability. LC exhibited relatively high chlorophyll-*a* concentrations but remained oxygen rich and only moderately nutrient loaded. The elevated productivity likely reflects strong photosynthetic activity and water circulation rather than external nutrient enrichment. LC represents a productive yet well oxygenated system, contrasting with the nutrient enriched, oxygen depleted conditions observed in HM and VP. Summer Chl-*a* peaks corresponded with optimal algal growth, reinforcing as a strong predictor of algal biomass (ECCC, 2023). Overall, LM showed stable chemistry and serves as a baseline wetland, while LC, VP, and HM exhibited variable nutrient enrichment tied to human activity.

3.4.2 Principal Component Analysis and Interpretation

A Principal Component Analysis (PCA) was conducted to examine multivariate patterns in the environmental data. The first two components explained 67.8% of total variance, with PC1 (49%) represented on the vertical axis and PC2 (18.8%) on the horizontal axis (Figure 3.1). PC1 represented a dissolved oxygen versus phosphorus/productivity gradient, with strong positive loadings for DO and strong negative loadings for TP, along with contributions from water temperature, chlorophyll-*a*, and conductivity. Wetlands with higher PC1 scores-led by LC, followed by LM exhibit more oxygenated, lower TP conditions. Wetlands with lower PC1 scores, including VP and HM, correspond to higher TP, warmer conditions, and greater productivity.

PC2 reflected a nutrient and organic matter gradient, dominated by strong positive loadings for TN and DOC, and negative associations with pH and conductivity. Wetlands located on the right side of PC2 (e.g., VP) display elevated TN and DOC concentrations, while wetlands on the left side (e.g., LC) show lower nutrient and DOC levels consistent with less impacted conditions.

The pattern observed along PC1 where high DO opposes elevated phosphorus, warm temperatures, and increased productivity, is consistent with other studies in freshwater systems. Studies across temperate wetlands and shallow lakes have shown that increases in phosphorus often lead to higher phytoplankton biomass, which reduces water clarity and decreases dissolved oxygen as respiration and decomposition intensify (Filstrup & Downing, 2018; Søndergaard et al., 2020). The separation of LC and LM from VP and HM along PC1 therefore mirrors nutrient oxygen patterns widely reported in literature.

In PC2, the strong positive associations between TN and DOC have been documented in wetlands influenced by watershed inputs, hydrologic connectivity, and organic matter decomposition (Creed et al., 2018; Xenopoulos et al., 2021). Similar PCA based wetland assessments have found that TN and DOC frequently load together, distinguishing more nutrient-enriched or organically influenced sites from clearer, less impacted systems (Zhang et al., 2022; Landesman & Parker, 2021). The placement of VP at the TN/DOC rich end of PC2, and LC at the opposite end, reflects patterns consistent with regional wetland studies showing that nutrient and organic matter enrichment often occur together in more disturbed water systems.

3.5 Conclusion

This chapter examined spatial and seasonal water quality variation across four Lake Simcoe wetlands. Each wetland displayed a distinct chemical profile shaped by both natural processes and anthropogenic pressures. LC had the highest DO and pH, consistent with active photosynthesis and strong circulation. HM and LM were intermediate, while VP showed the lowest DO and pH, indicating organic enrichment and limited mixing. HM exhibited the highest conductivity, reflecting agricultural ion inputs; VP had the lowest conductivity but most variable. TN and TP were lowest in LC and highest in HM and VP, indicating nutrient loading gradients. DOC was greatest in VP, aligning with high organic matter decomposition, while Chl-*a* was highest in VP and second highest in LC - likely due to high light availability in LC's clear waters and active photosynthesis, rather than nutrient loading that we see in VP.

Although seasonal variation influenced water quality within each site, spatial differences among wetlands explained a greater proportion of the overall variance. PCA results indicated that oxygenation and nutrient gradients between wetlands were stronger structuring forces than temporal fluctuations, emphasizing that site level characteristics dominated over seasonal effects.

LC represented a well oxygenated, low nutrient system; LM showed stability; HM and VP reflected nutrient enrichment and reduced oxygenation. These findings provide a clear framework for identifying nutrient driven stress in wetlands and underscore the value of combining multivariate analysis with seasonal monitoring for management of the Lake Simcoe watershed.

Chapter 4 : The Influence of Wetland Health on Epiphyte Community Structure: A Cumulative Study

4.1 Introduction

Bioindicators are organisms or biological communities used to assess the quality of an environment and detect changes over time due to factors such as pollution, habitat loss, climate change, hydraulic changes, etc. These environmental changes can be physical, chemical, or biological in nature (Milosevic et al., 2020; Agersted et al., 2021). Recent comparative assessments of freshwater monitoring approaches have emphasized that no single taxonomic group, whether benthic macroinvertebrates, algae, fish, or zooplankton - functions as a universally superior bioindicator. Instead, each group responds to different stressors and ecological gradients, making their usefulness dependent on the habitat type, study objectives, and the specific pressures acting on an ecosystem (Kelly et al., 2020; Birk et al., 2020). These findings reinforce the importance of selecting bioindicator taxa that align with the characteristics and monitoring needs of individual freshwater environments. In shallow, vegetated wetland environments such as those found across the Lake Simcoe watershed in Ontario, algae are particularly well suited for use as bioindicators. They exhibit wide spatial and temporal distribution, occur in large and diverse populations, are easy to collect and identify, and often exhibit genus/species specific responses to stressors (Ponader & Charles, 2021; Schneider et al., 2021).

Among epiphytes, certain species are associated with high nutrient or otherwise polluted conditions, while others thrive only in clean, or oligotrophic systems. Pollution tolerant taxa often dominate under conditions of eutrophication, low oxygen, and high turbidity. These include *Euglena* spp., *Nitzschia* spp., *Navicula* spp., and *Scenedesmus* spp. All of which have

been documented in elevated abundance in nutrient rich environments. For instance, many species of *Euglena* spp. and *Nitzschia* spp. are known to thrive in environments with high organic loading and degraded water quality (Al-asadi et al., 2020).

In contrast, species such as *Fragilaria* spp., *Gomphonema* spp., *Synedra ulna*, and *Staurosira construens,* are more sensitive to pollution and typically flourish in clearer, less nutrient impacted systems. These epiphytes are often among the first to decline when the water quality deteriorates, making them reliable indicators of ecological stress (Stevenson et al., 2010).

Epiphytes play a critical ecological role by contributing to primary production, serving as a food source for grazers, influencing nutrient cycling and DO, and controlling energy flow in freshwater systems (Garcia et al., 2020; Lyu et al., 2022; Zhang et al., 2023). In monitoring wetland health, epiphytes are particularly important because of their short life cycles, and sensitivity to changes in water quality and habitat conditions. Because they are constantly and directly exposed to changing environmental gradients such as sunlight availability, nutrient loading, aquatic pollution, and water chemistry changes, this in combination with their short life cycles, they are considered as good candidates for monitoring the environmental changes quickly as well as over time (Lange et al., 2021; Schneider et al., 2023). This chapter investigates how variations in the water quality parameters influence epiphyte populations and community structure across the four selected wetlands in the Lake Simcoe watershed.

4.2. Methods

The description of methods of Macrophyte and epiphyte collection and data preparation is given in Chapter 2 (Materials and Methods). The following section describes the results of the sample analyses and the statistical methods used.

Statistical Analysis

To examine spatial and temporal variation in epiphytic algal communities, a combination of statistical analyses was employed. Univariate analyses were first used to test whether basic community metrics, specifically Shannon Diversity Index (SDI), species richness, and epiphyte density, differed significantly across wetlands and among seasons. These metrics were selected because they provide complementary perspectives and a deeper understanding on community structure. SDI incorporates both richness and evenness into a single measure of diversity (Shannon, 1948), species richness captures the number of taxa present, and density reflects total algal abundance. This is equally important in understanding the health of an epiphyte community. A two-way analysis of variance (ANOVA) was applied to each of these metrics with wetland and season as fixed factors, enabling the assessment of both main effects and potential interaction effects between spatial and temporal variability (Quinn & Keough, 2002). This approach was chosen because it allows for a direct comparison of community characteristics across sites and sampling periods while controlling for variation attributable to each factor. When significant main effects were identified in the ANOVA models, Tukey's Honest Significant Difference (HSD) post hoc tests were conducted to determine the specific wetland or seasonal pairs that accounted for significant differences (Sokal & Rohlf, 1995). Post hoc testing was particularly important in this study, as it allowed for the detection of which individual comparisons drove significant patterns in the data. ANOVAs were run on the densities of the most abundant epiphytic taxa to test whether dominant species showed site level variation in abundance or seasonal level variation. This analysis provided insight into whether observed patterns in community metrics were attributable to broad community changes within wetlands due to water quality or if there were similar patterns across all wetlands sampled.

A Principal Component Analysis (PCA) was performed on the combined biological and environmental dataset to reduce dimensionality and to visualize major gradients in the structure of epiphyte assemblages (Legendre & Legendre, 2012). A PCA was selected because it provides a means of identifying which environmental variables are most strongly associated with patterns in richness, density, and diversity. Finally, a Permutational Multivariate Analysis of Variance (PERMANOVA) was used on the community composition data to formally test whether overall epiphyte community structure differed significantly among wetlands as well as seasons (Anderson, 2001). The PERMANOVA was selected because it is a robust, nonparametric method that does not rely on assumptions of multivariate normality and is particularly appropriate for ecological community data.

These methods provided the statistical foundation for evaluating how epiphyte diversity, density, and species richness vary across wetlands and seasons, and for linking those patterns to underlying environmental conditions.

Phytoplankton data were analyzed using both univariate and multivariate statistical approaches. Mean cell densities, PSD, species richness, and diversity indices were calculated for each site and sampling period (Magurran, 2013). Differences among wetlands and seasons were tested using one-way and two-way ANOVA, with Tukey's post hoc comparisons where appropriate (Zar, 2010). Assumptions of normality and homogeneity of variance were verified using Shapiro-Wilk and Levene's tests (Shapiro & Wilk, 1965; Levene, 1960). Community composition patterns were examined through nonmetric multidimensional scaling (NMDS) based on Bray-Curtis similarities (Bray & Curtis, 1957), and significance among groups was evaluated using PERMANOVA (Anderson, 2001). Indicator species analysis was used to identify taxa characteristic of individual wetlands (Dufrêne & Legendre, 1997).

All statistical tests were conducted in R (R Core Team, 2024) using the R-Studio integrated development environment (R-Studio Team, 2023).

4.3 Results

4.3.1 Epiphyte Species Diversity

The total number of epiphytes species observed during the study from four wetlands include LC with 68, HM with 56, LM with 55, and VP with 50. The results of a two-way ANOVA of the Shannon Diversity Index (SDI) calculations indicated that epiphyte diversity differed significantly both between wetlands ($p < 0.05$) and between seasons ($p < 0.05$). Tukey's post hoc analysis revealed that seasonal differences in diversity were driven primarily by significant variation between summer and fall. Pairwise comparisons between wetlands showed significant differences in diversity index between LC and HM, VP and HM, VP and LC, and VP and LM. When examined by season with a post hoc test, fall samples showed significant differences between HM and VP, LC and VP, as well as LM and VP. In spring, only the LC- VP comparison was significant. During summer, all wetlands showed significantly higher diversity when comparing to VP. HM and LM had similar average SDI measurements, although HM had a much higher SD (Figure 4.1). Between seasons, the average SDI calculations were highest in the fall (2.45), followed by spring (2.33) and lowest in the summer (2.29).

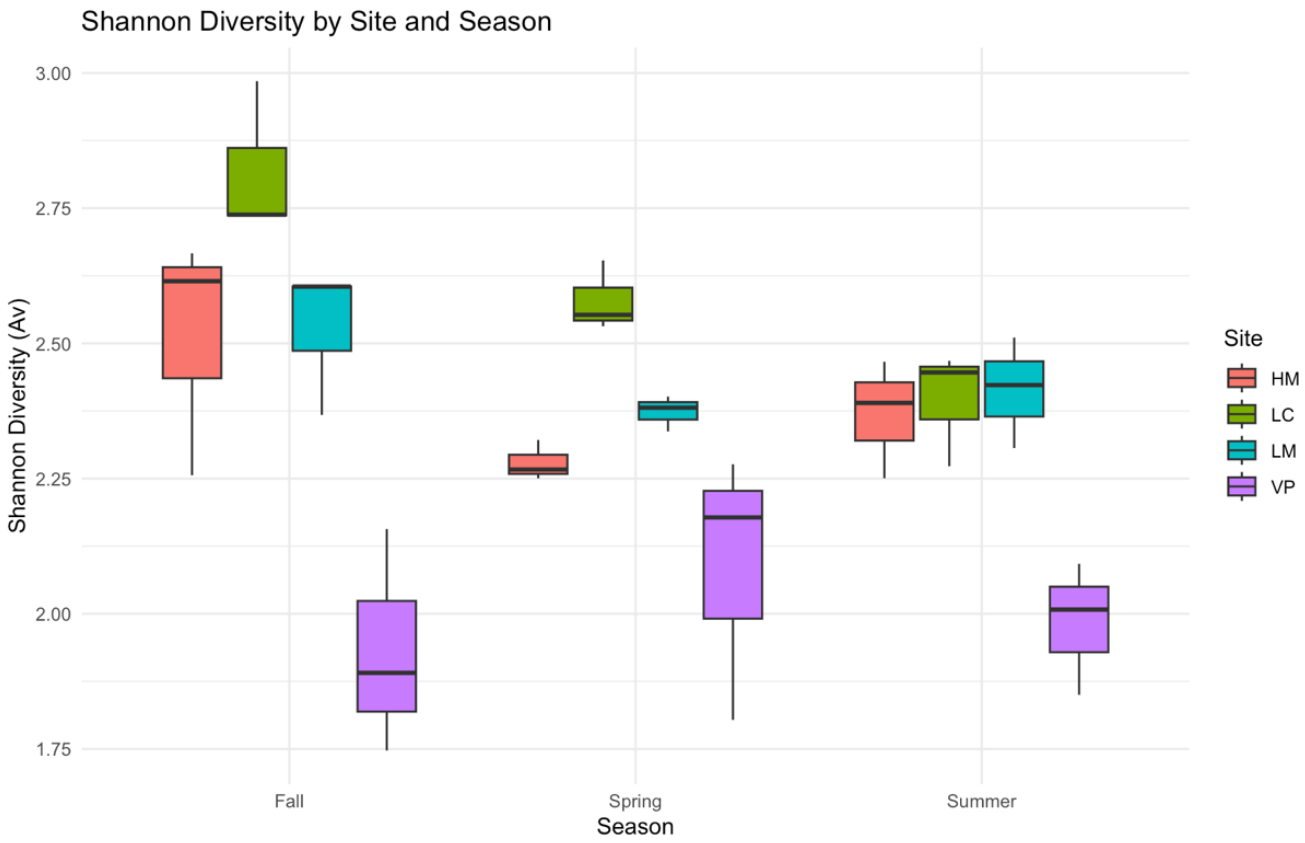


Figure 4.1 - Box Plot Graph of Shannon Diversity Index of Epiphytes by Wetland (HM, LC, LM, VP) and Season. Boxplots show medians and variability across wetlands and seasons.

4.3.2 Density

The two-way ANOVA results measured the epiphyte density (cells/mm ^{2}) differing significantly between wetlands sampled ($p < 0.05$) as well as between seasons ($p < 0.05$). Tukey's post hoc analysis identified significant differences in density between LC and HM, VP and HM, VP and LC, and VP and LM. Seasonal comparisons revealed a significant difference in density between spring and fall only (Table 4.1).

Although VP exhibited the highest chlorophyll-*a* concentrations among the sampled wetlands, it also measured the lowest epiphyte richness, diversity, and density. These results reflect the importance of including phytoplankton in these preliminary comparative studies. In nutrient and organic rich systems such as VP, elevated TP, TN, and DOC, these factors can

promote phytoplankton blooms. These blooms will increase chlorophyll-*a*, while reducing DO and light penetration in the water. Due to this growth-related turbidity, shading is increased which inhibits the growth of attached algal forms, leading to low epiphyte density despite high overall algal productivity and phytoplankton density. These chlorophyll-*a* values at VP should be interpreted primarily as evidence of possible eutrophication rather than epiphytic abundance.

LC and HM wetland exhibited the highest standard deviation in epiphyte density (LC = 694.45 and HM = 610.35), reflecting substantial variability across samples (Table 4.2). This elevated variability can be attributed to the wide range of density values recorded within the site, spanning from density calculations as low as 100 cells/mm ^{2} to over 2,000 cells/mm ^{2}.

Cumulative epiphyte density varied across wetlands and seasons. Based on seasonal averages (Table 4.3), LC consistently measured the highest cumulative densities, particularly in the fall (1622 cells mm $^{-2}$), while VP maintained the lowest values across all seasons, with a minimum of 205 cells mm $^{-2}$ in the fall. While LM and HM measured intermediate densities, with LM peaking in the fall (970.1 cells/mm ^{2}) and HM measuring the highest in the summer (948.4 cells/mm ^{2}).

When considering total cumulative density (Table 4.4), the same pattern was observed with LC recording the greatest values overall (28,181.5 cells/mm ^{2}) and VP the lowest (13,427.7 cells/mm ^{2}). LM and HM again occupied intermediate positions (19,987.3 cells/mm ^{2} and 20,407.7 cells/mm ^{2}, respectively). Across wetlands, fall contributed the highest cumulative densities, while spring generally produced the lowest.

Table 4.1 - Average Density of Epiphytes by Season

Season	Average Density of Epiphytes (cells/mm ^{2})	Standard Deviation
Spring	490.90	357.50
Summer	683.14	409.68
Fall	986.27	786.61

Table 4.2 - Average Density of Epiphytes by Wetland

Wetland	Average Density of Epiphytes (cells/mm ^{2})	Standard Deviation
Lagoon City	1062.15	694.45
Langman's Marsh	763.57	472.02
Holland Marsh	734.70	610.35
Victoria Point	319.99	190.14

Table 4.3- Cumulative Seasonal Averages of Epiphyte Density by Wetland

Season	Langman's Marsh	Lagoon City	Holland Marsh	Victoria Point
Spring	536	666.4	179.1	302.2
Summer	481.9	602.7	948.4	798
Fall	970.1	1622	903.3	205
Average Annual	662.67	963.70	676.93	435.07

Table 4.4 - Cumulative Total Epiphyte Density by Wetland and Season

Season	Langman's Marsh	Lagoon City	Holland Marsh	Victoria Point
Spring	15108.3	19932.7	6285.7	9720.6
Summer	15698.3	18244.5	28486.3	24094.2
Fall	29155.2	46367.3	26451	6468.3
Annual Average	19987.27	28181.50	20407.67	13427.70

4.3.3 Species Richness

The results of a two-way ANOVA revealed that average epiphyte species richness per wetland varied significantly with site ($p < 0.05$) and season ($p < 0.05$). Tukey's HSD post hoc test identified significant pairwise differences in species richness between the following site combinations: LC-HM, VP-HM, VP-LC, and VP-LM. LC exhibited the highest average species richness (24.85), followed by LM (20.85), HM (18.63), and VP (12.74) (Table 4.5). The highest

total number of species was in LC (68), followed by LM (55), HM (56), and lowest at VP (50) (Table 4.6). A full list of species per wetland found in Table 4.7.

Table 4.5 Average Species Richness of Epiphytes by Wetland

Location	Average Species Richness	Standard Deviation
Lagoon City	24.85	7.17
Langman's Marsh	20.85	6.21
Holland Marsh	18.63	6.07
Victoria Point	12.74	3.62

Table 4.6 - Total Number of Epiphyte Species Identified Per Wetland

Location	Identified Species by Genus
Lagoon City	68
Langman's Marsh	55
Holland Marsh	56
Victoria Point	50

Table 4.7- Epiphytic algal genera observed across the four wetlands. Presence at each site is indicated by a '+' symbol, with algal group indicated by color. Blue/diatoms, green/green algae, pink/dinoflagellates, orange/euglenoids, & yellow/yellow-green algae

Epiphyte Genus	**Holland Marsh**	**Langman's Marsh**	**Lagoon City**	**Victoria Point**
Achnanthes spp.	+	+	+	+
Amphora spp.	+	+	+	+
Ankistrodesmus spp.	+	+	+	+
Anomoeoneis spp.			+	
Asterionella formosa		+	+	
Aulacoseira spp.		+	+	
Bulbochaete spp.	+	+	+	+
Ceratium spp.	+			
Carteria spp.				+
Chlamydomonas reinhardtii	+	+	+	+
Chlamydomonas spp.	+	+	+	+
Chlorella spp.	+	+	+	+
Cladophora spp.	+	+	+	+
Closterium spp.		+	+	
Cocconeis spp.	+	+	+	+

Coelastrum spp.	+	+	+	+
Cosmarium spp.	+	+	+	+
Craticula cuspidata			+	+
Craticula halophila			+	
Craticula spp.	+		+	+
Cyclotella spp.	+	+	+	+
Cylindrocystis spp.				+
Cymbella spp.	+	+	+	+
Denticula spp.	+		+	+
Desmodesmus spp.	+	+	+	+
Diatoma spp.	+	+	+	+
Diatoma vulgaris	+		+	
Epithemia spp.			+	
Eudorina spp.	+	+	+	+
Euglena spp.	+	+	+	+
Eunotia arcus	+	+	+	+
Eunotia fennica	+	+	+	+
Eunotia spp.	+	+	+	+
Fragilaria spp.	+	+	+	+
Gomphonema spp.	+	+	+	+
Gymnodinium spp.	+	+	+	+
Haematococcus spp.	+		+	+
Hydrodictyon spp.		+	+	
Kirchneriella spp.			+	
Lepocinclis spp.	+		+	
Melosira granulata	+	+	+	+
Navicula spp.	+	+	+	+
Neidium affine	+	+	+	+
Nephrocytium spp.			+	
Nitzschia acicularis	+	+		
Nitzschia nana				+
Nitzschia spp.	+	+	+	+
Oedogonium spp.	+	+	+	+
Oocystis spp.	+	+	+	+
Pediastrum boryanum var. cornutum	+	+	+	
Pediastrum spp.	+	+	+	+
Peridinium spp.	+	+	+	
Phacus spp.	+	+	+	+
Pinnularia spp.	+	+	+	+
Pleurococcus spp.		+	+	+

Rhoicosphenia spp.	+	+	+	+
Rhopalodia gibba	+	+	+	+
Scenedesmus spp.	+	+	+	+
Sphaerocystis spp.		+		
Spirogyra spp.	+		+	
Spondylosium pulchellum			+	
Staurastrum convergens			+	
Staurastrum spp.		+	+	
Staurosira construens		+		
Stephanodiscus spp.	+	+	+	+
Stigeoclonium spp.	+	+	+	+
Synedra famelica		+	+	
Synedra spp.	+	+	+	+
Synedra ulna	+	+	+	+
Tetrabaena spp.			+	
Tetraedron spp.	+		+	+
Tetraspora spp.	+	+	+	
Trachelomonas spp.	+		+	
Tribonema spp.	+			
Ulothrix spp.	+	+		
Volvox spp.	+	+	+	
Zygnema spp.	+	+	+	+

4.3.4 Per Species Density (PSD) of 15 most common Epiphyte Species

A PERMANOVA detected significant differences in per species density (PSD) among wetlands ($p < 0.05$). Pairwise comparisons confirmed that all wetlands differed significantly from one another ($p < 0.05$). A multilevel pattern analysis was used to discern any significant differences between the exclusiveness or frequency of each of the top 15 species. This test identified significant indicator taxa across wetlands ($p < 0.05$). Figure 4.2 displays non-ubiquitous species, defined as taxa restricted to one or two wetlands only, further emphasizing the community differences between wetlands.

The following are the species found only in HM: *Ceratium* spp., *Rhopalodia gibba*, *Chlamydomonas reinhardtii*, *Tribonema* spp., and *Melosira granulate*. In VP: *Chlamydomonas*

carteria, *Cylindrocystis* spp.*,* and *Tabellaria fenestrate*. In LM: *Rhopalodia* spp.*, Sphaerocystis* spp.*,* and *Staurosira construens*. And in LC: *Anomoeoneis* spp.*, Craticula* spp.*, Eudorina* spp.*, Euglena* spp.*,* and *Snowella* spp. By contrast, several taxa were recorded in all four wetlands and also ranked among the fifteen most common species, they include *Nitzschia* spp., *Navicula* spp., *Eunotia* spp., *Synedra* spp., and *Cymbella* sp (Figure 4.2).

Relative abundance plots revealed that *Navicula* spp. and *Eunotia* spp. were consistently the top two most abundant genera across all wetlands, although secondary contributors varied by site. In HM, *Cocconeis* spp., *Cymbella* spp.*, Eunotia* spp.*, Navicula* spp.*, Rhoicosphenia* spp.*,* and *Synedra* spp.*,* were prominent (Figure 4.3). LC was characterized to have similar larger populations of species mentioned above, as well as *Achnanthes* spp. and *Rhopalodia gibba.* (Figure 4.4). At VP, where the least variability of species and densities observed *Bulbochaete* spp.*, Eunotia* spp., *Navicula* spp.*, Nitzschia* spp.*, Pinnularia* spp.*,* and *Synedra* spp. accounting for the highest proportion overall genera (Figure 4.5). LM showed even distribution among species, *Bulbochaete* spp.*, Eunotia fennica, Eunotia* spp.*, Fragilaria* spp.*, Gomphonema* spp.*, Navicula* spp.*, Nitzschia* spp.*,* and *Synedra* spp.*,* and *Synedra ulna.* (Figure 4.6).

Comparisons of the top 15 species per wetland confirmed site specific differences. LC exhibited the greatest taxonomic evenness, while VP displayed strong dominance by a few taxa (notably *Eunotia* spp. and *Navicula* spp.*)* (Figure 4.7). Seasonal assessments further indicated dynamic shifts in species composition. For example, in LC, *Eunotia* spp. and *Nitzschia* spp. were most abundant in the spring but declined relative to *Cymbella* spp. in the summer, and *Achnanthes* spp. was the most abundant in the spring and the fall. At VP, *Nitzschia* spp. and *Eunotia* spp. were more common in spring but were replaced by *Navicula* spp. in the summer and the fall (Figure 4.8).

When comparing wetlands, LC consistently supported the greatest species richness and evenness, whereas VP showed the lowest diversity and strongest dominance patterns (Figure 4.9). HM and LM fell between these two extremes, exhibiting intermediate levels of richness and abundance balance. LM and HM supported a similar number of species, but LM supported larger and more even distribution among the populations of each species compared to HM.

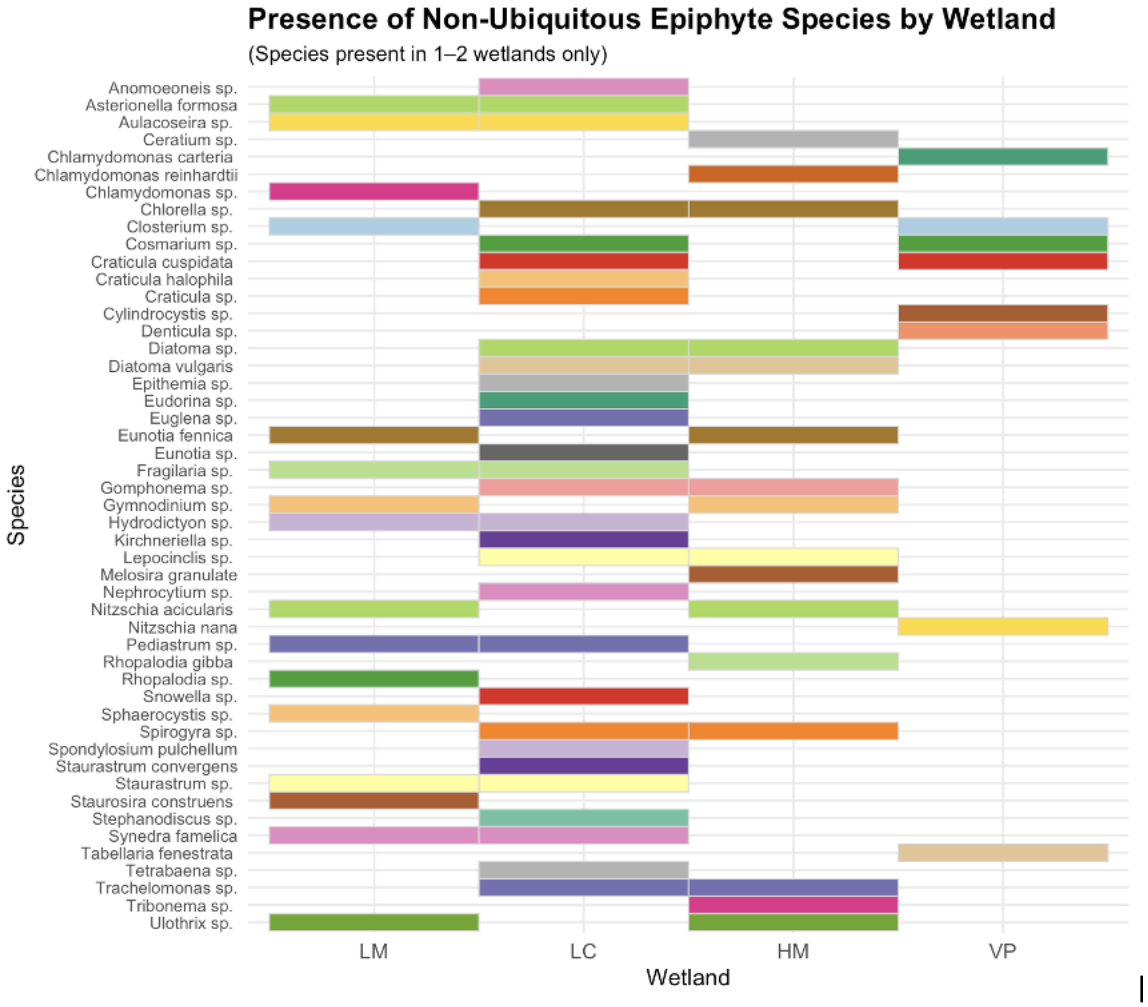


Figure 4.2 - Presence of Non-Ubiquitous Epiphyte Species by Wetland. Distribution of epiphyte species occurring in only one or two wetlands. Bars indicate species presence within LC, LM, HM, and VP.

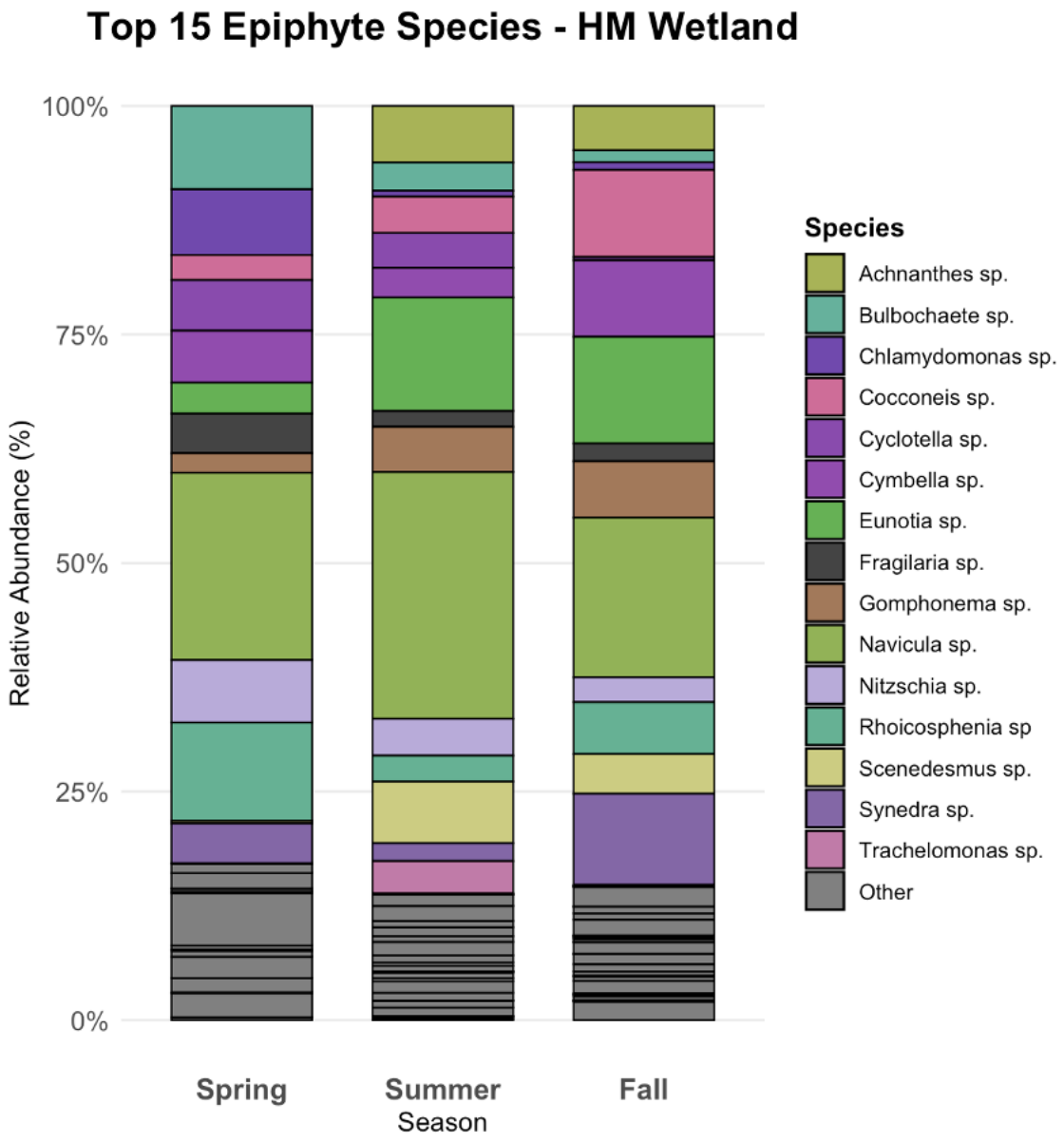


Figure 4.3 - Relative abundance of the top 15 epiphyte species in HM across spring, summer, and fall. Navicula spp.*, Eunotia* spp.*, and Cocconeis* spp. *were consistently present, with higher variability across seasons.*

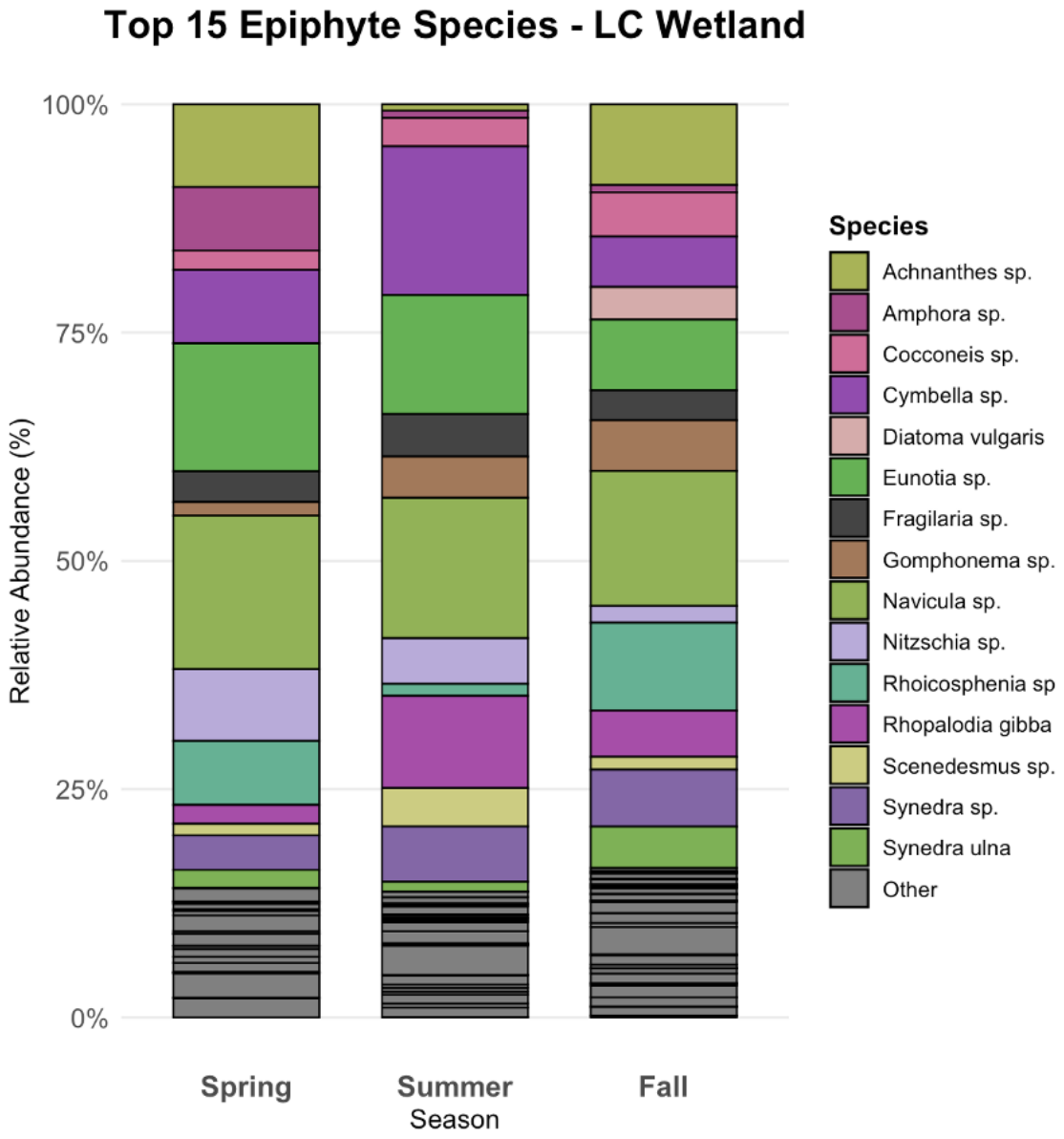


Figure 4.4 - Relative abundance of the top 15 epiphyte species in LC across spring, summer, and fall. Community composition shifts seasonally, with several dominant taxa including Achnanthes spp.*, Navicula* spp.*, Eunotia* spp.*, and Cymbella* spp.

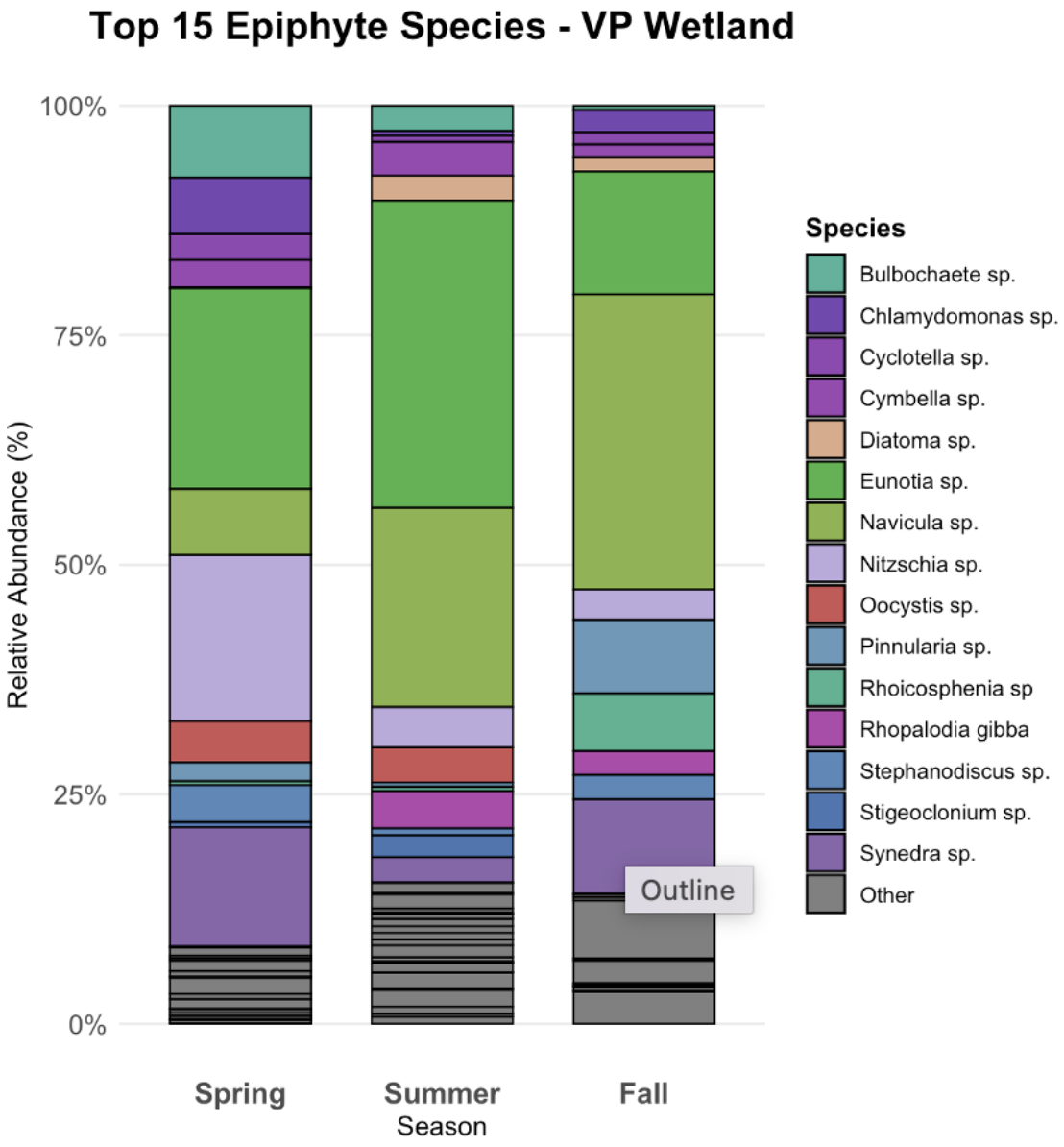


Figure 4.5 - Relative abundance of the top 15 epiphyte species in VP across spring, summer, and fall. VP communities were strongly dominated by Eunotia spp. *and Navicula* spp.*, with a smaller number of other taxa contributing proportionally to community structure.*

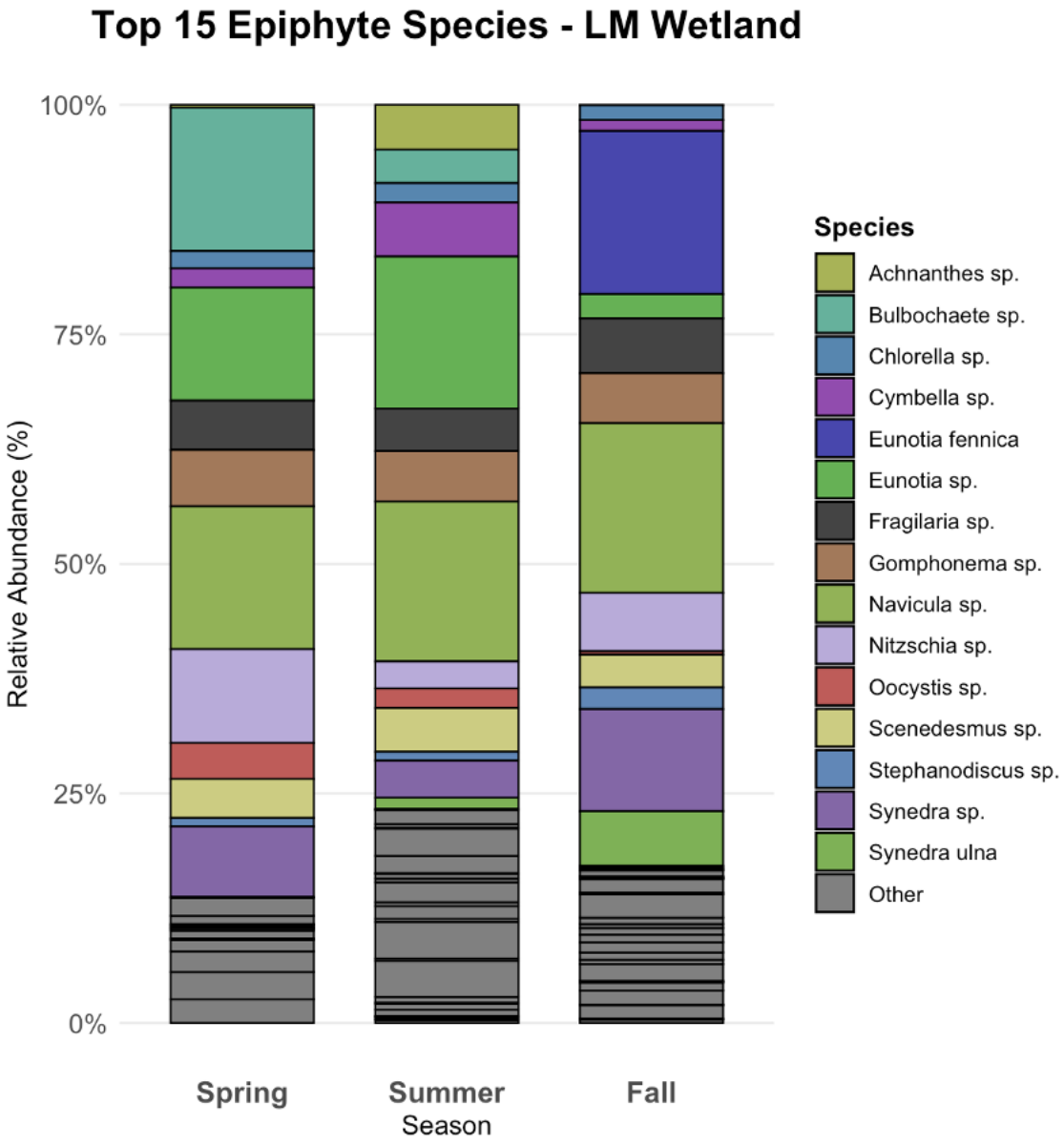


Figure 4.6 - Relative abundance of the top 15 epiphyte species in LM across spring, summer, and fall. Navicula spp. *and Eunotia* spp. *were consistently abundant, but additional taxa such as Fragilaria* spp. *and Gomphonema* spp. *also contributed to community composition.*

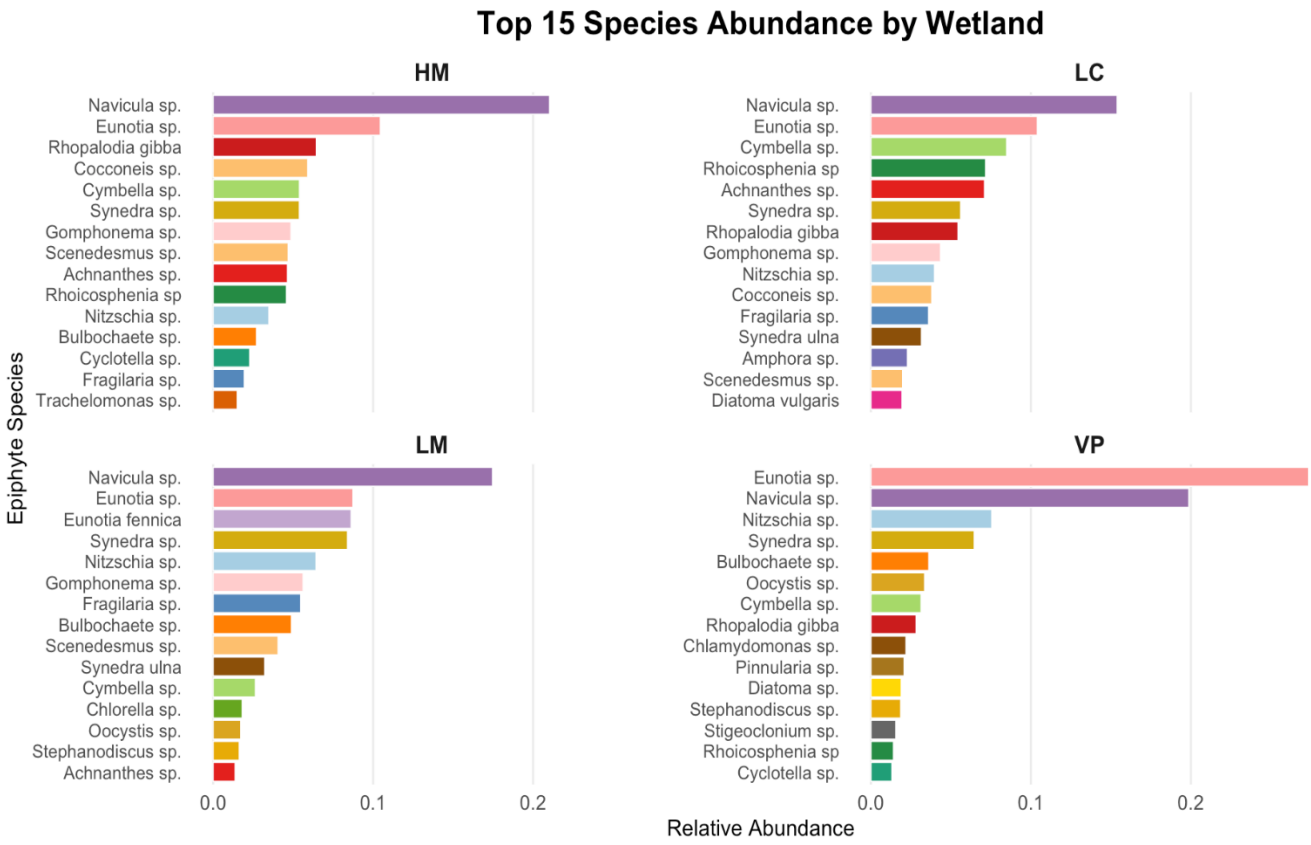


Figure 4.7 - Relative Abundance of the Most Abundant Epiphyte Species by Wetland. Relative abundance of the 15 most dominant epiphyte species in each wetland (HM, LC, LM, VP).

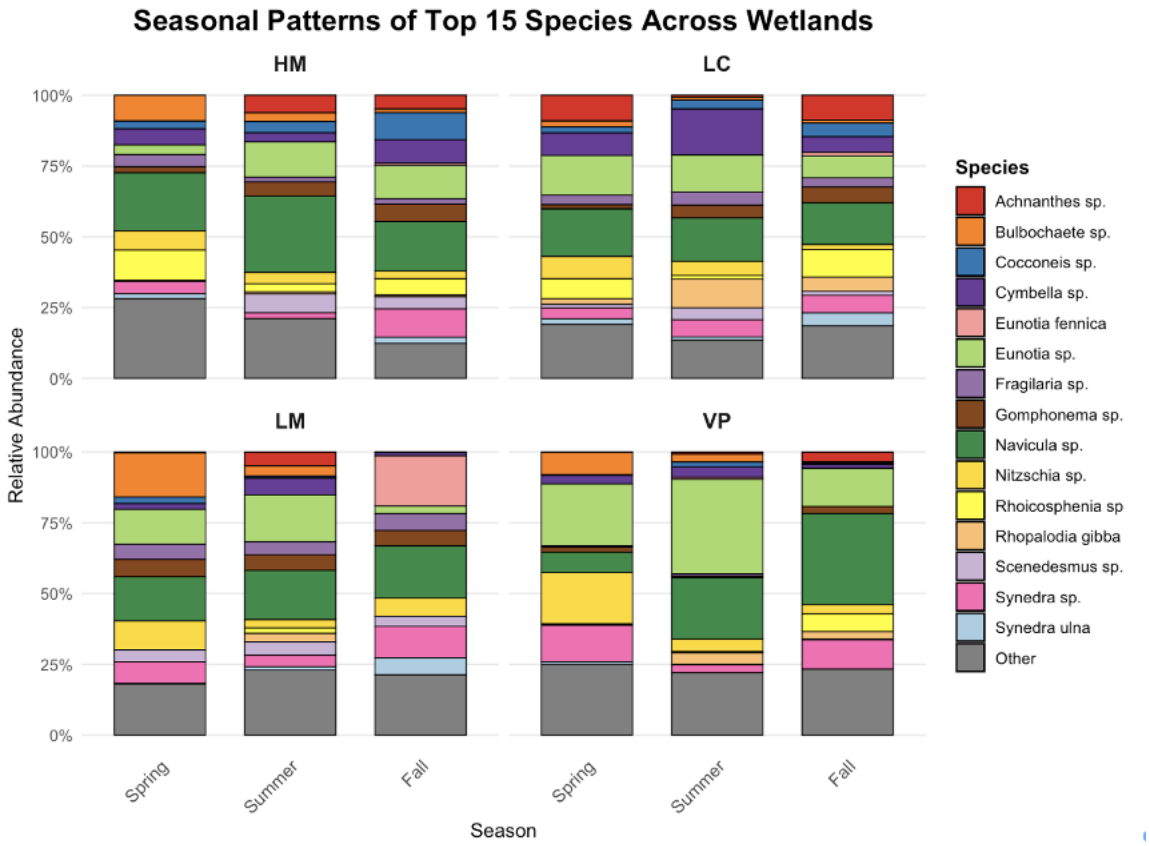


Figure 4.8 - Figure 4.8 Seasonal Patterns in Dominant Epiphyte Species Relative Abundance by Wetland and Season. Seasonal variation in the relative abundance of the top 15 epiphyte species across wetlands. The plots illustrate shifts in community composition.

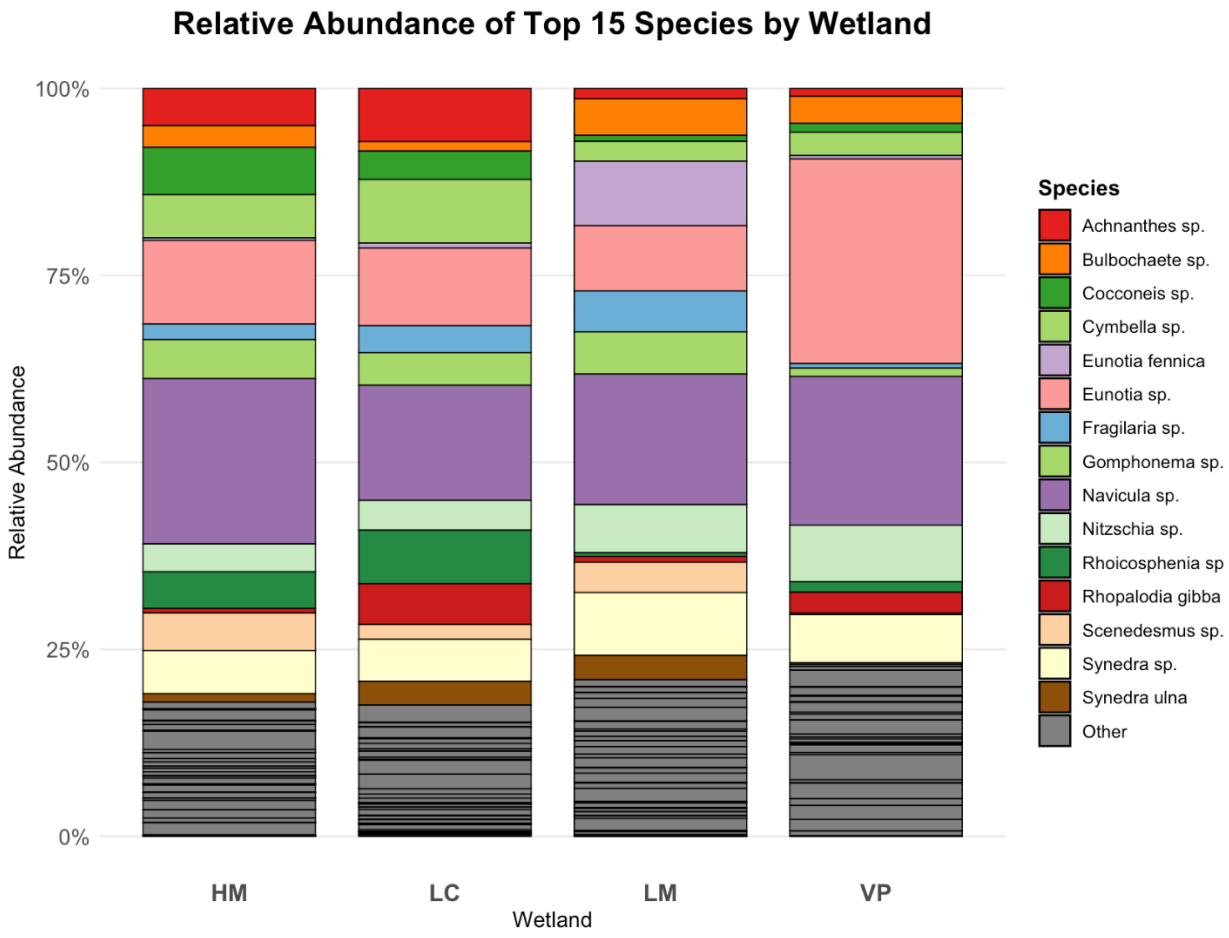


Figure 4.9 - Abundance of Top 15 Species by Wetland. Stacked bar chart showing the relative abundance (%) of the top 15 epiphyte species across wetlands. Species such as Navicula spp.*, Eunotia* spp.*, and Cymbella* spp. *were consistently dominant.*

4.3.5 Wetland Performance Scores

Wetland performance scores were calculated by integrating biological integrity with water quality indicators. Biological scores reflected species richness, the balance between general tolerant and sensitive taxa, and the stability of assemblages. Water quality scores reflected nutrient concentrations, conductivity, and related metrics. Each component was scaled to 0 to 100, and the two components (biological scores and water quality scores) were averaged to create a comprehensive index (Stoddard et al., 2008; U.S. EPA, 2002).

The combined wetland performance score was applied as a practical means of integrating multiple dimensions of wetland condition into a single measure. Because wetlands are influenced by a wide range of biological and environmental variables, focusing only on one dimension risks giving an incomplete assessment of ecosystem health. Multi-metric approaches address this by incorporating both biological integrity and abiotic context, providing a more balanced evaluation of overall condition (Stoddard et al., 2008; U.S. EPA, 2002). To facilitate comparison across sites, each component was standardized to a 0-100 scale. This method is consistent with established indices such as the Ohio Vegetation Index of Biotic Integrity (VIBI), which demonstrates how biological metrics can be normalized for broader application (Mack, 2007). By averaging biological and water quality scores, the resulting index aligns with accepted monitoring frameworks while remaining good enough to be applied across diverse wetland types (Chidiac et al., 2023).

Comprehensive scores ranked LC the highest (90.7), followed by LM (75.8). HM (66.6) and VP (66.3) the lowest (Figure 4.10). When separated into biological and water quality components, LC performed strongly across both, while VP showed particularly poor biological integrity. Relative to the group mean, LC outperformed by +15.9 points, LM was near the average (+0.9), while HM and VP underperformed (−8.3 and −8.5) (Figure 4.11 and 4.12).

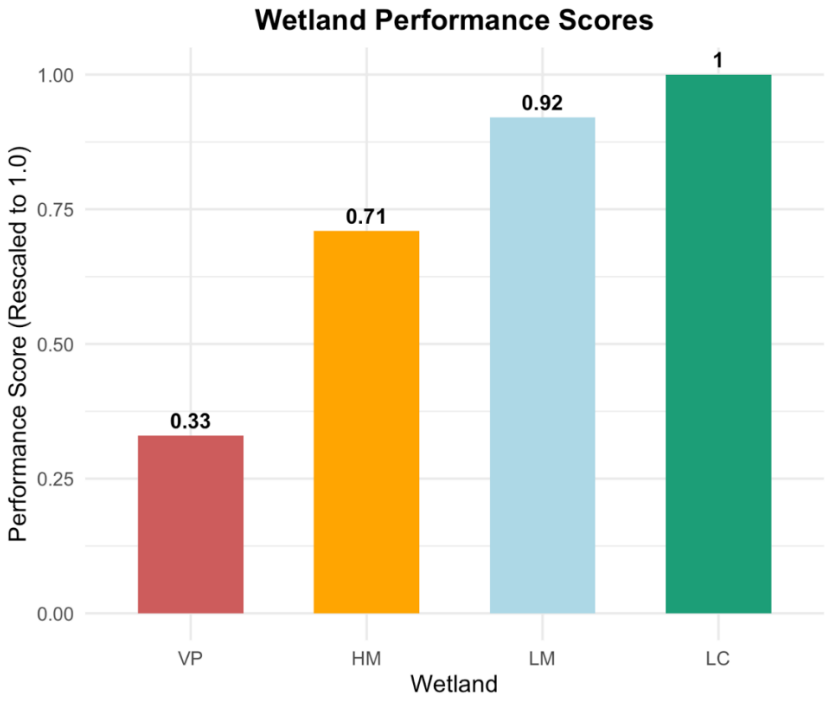


Figure 4.10 - Comparative Wetland Performance Scores by Site. The Bar chart shows rescaled wetland performance scores (0-1 scale) across sites. Scores integrate biological and water quality metrics to provide a comparative assessment of wetland condition.

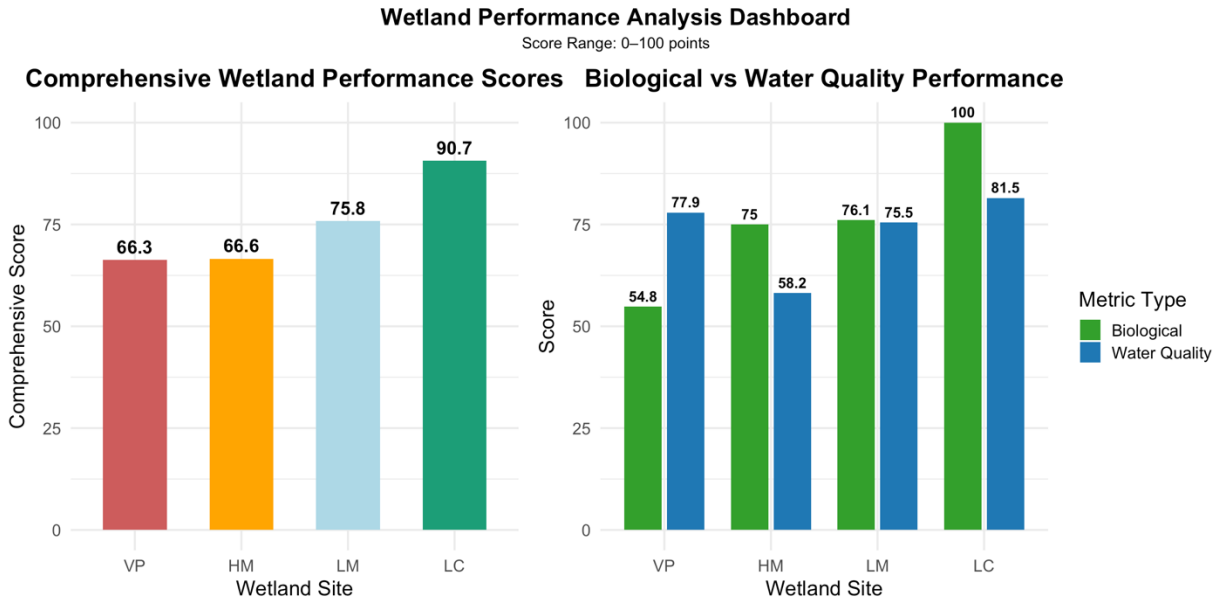


Figure 4.11 - Comprehensive wetland performance scores (left) and biological versus water quality performance scores (right). Comprehensive scores ranged from 66.3 at VP to 90.7 at LC.

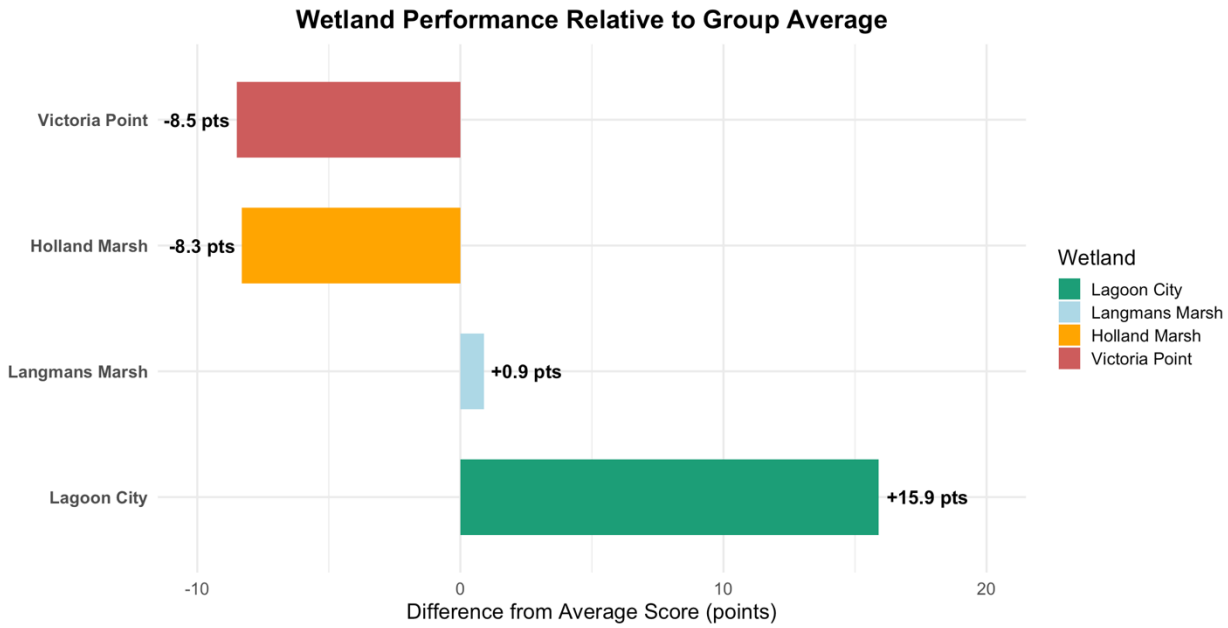


Figure 4.12 - Wetland Performance Relative to Group Average. Performance scores of wetlands expressed as differences from the group average. LC scored +15.9 points above the average, while VP (−8.5) scored the lowest below the group.

4.3.6 Phytoplankton

Phytoplankton Diversity

The average phytoplankton Shannon Diversity Index (SDI) measurement varied slightly among wetlands (Table 4.8). LM had the highest average SDI (2.61), followed by HM (2.55), VP (2.48), and LC (2.34). Across seasons (Table 4.9), the average phytoplankton SDI was highest in the spring (2.67), followed by the fall (2.49) and the summer (2.42). A two-way ANOVA was used to evaluate the differences in phytoplankton Shannon Diversity Index (SDI) among wetlands and across seasons. Results indicated that there were no statistically significant differences in SDI among wetlands or among seasons ($p > 0.05$).

Table 4.8 - Average Phytoplankton Shannon Diversity Index Across Wetlands

Wetland	Average Shannon Diversity Index
Holland Marsh	2.55
Langman Marsh	2.61
Lagoon City	2.34
Victoria Point	2.48

Table 4.9- Average Phytoplankton Shannon Diversity Index Across Seasons

Season	Average Shannon Diversity Index
Spring	2.67
Summer	2.42
Fall	2.49

Phytoplankton Density

Average phytoplankton density (cells/L) varied among wetlands and across seasons. Among wetlands, HM had the highest mean densities, approximately 4.06×10^6 cells/L, followed by LM at 3.54×10^6 cells/L, VP at 2.73×10^6 cells/L, and LC at 2.68×10^6 cells/L. Across seasons, mean densities were highest in the summer (4.28×10^6 cells/L), decreased during the fall (3.28×10^6 cells/L), and were lowest in the spring (2.20×10^6 cells/L) (Figure 4.13).

A two-way ANOVA was performed to assess differences in phytoplankton density among wetlands and across seasons. Results indicated that wetlands did not differ significantly in overall phytoplankton density ($p > 0.05$). A significant seasonal effect was observed ($p < 0.05$), after using a Tukey comparison, it was revealed that there was a significant difference in density between spring and summer only.

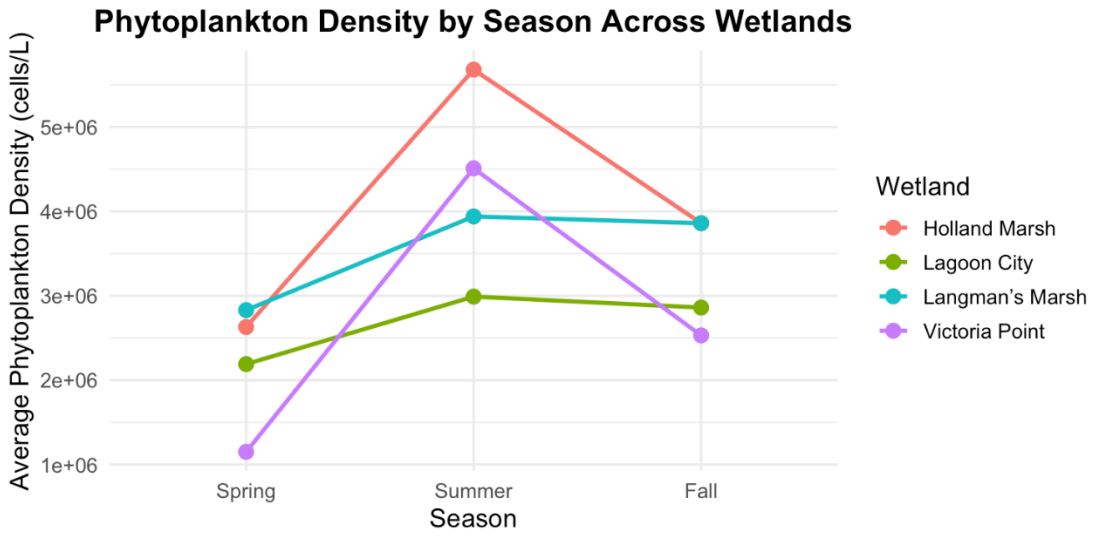


Figure 4.13 - A visualization of the change in phytoplankton density in each wetland by each season.

Phytoplankton Species Richness

Average richness values were highest at HM which contained 26 taxa in the spring, 19 in the fall, and 17 in the summer. LM maintained relatively high richness (17, 14, and 21 taxa across the three seasons). LC and VP consistently exhibited lower phytoplankton richness, each ranging from 13 - 16 taxa (Table 4.10). The Phytoplankton species per wetland were, HM at 37, LM at 32, VP at 32, and LC at 21 (Table 4.11). A list of species found at each wetland can be seen in Table 4.12. A two-way ANOVA was used to compare phytoplankton richness among wetlands and across seasons. Results indicated that there were no statistically significant differences in species richness among wetlands or among seasons ($p > 0.05$).

Table 4.10 - Phytoplankton Species Richness by Season and Wetland

Wetland	Season		
	Spring	Summer	Fall
Holland Marsh	26	17	19
Lagoon City	14	13	15
Langman's Marsh	17	14	21
Victoria Point	14	16	16

Table 4.11- Phytoplankton Species Richness per wetland

Wetland	Richness
Holland Marsh	37
Lagoon City	21
Langman's Marsh	32
Victoria Point	32

Table 4.12 - Phytoplankton by major algal groups at each site, indicated by '+', with algal group indicated by color - dinoflagellates/pink, diatoms/blue, green algae/green, euglenoids/orange, yellow-green algae/yellow and golden-brown algae/aqua.

Phytoplankton Genus	**Holland Marsh**	**Langman's Marsh**	**Lagoon City**	**Victoria Point**
Achnanthes spp.	+	+	+	+
Amphora spp.	+	+	+	+
Ankistrodesmus spp.	+	+		
Anomoeoneis spp.	+		+	
Aulacoseira spp.	+	+		
Bulbochaete spp.	+	+		+
Chlamydomonas spp.	+	+	+	+
Chlorella spp.		+		
Cladophora spp.	+			
Cocconeis spp.	+		+	+
Cosmarium spp.		+	+	+
Craticula spp.	+			
Cyclotella spp.	+	+	+	+
Cymbella spp.	+	+	+	+
Desmodesmus spp.	+	+		
Diatoma spp.	+	+		+
Euglena spp.	+	+	+	+
Eunotia arcus		+		
Eunotia fennica		+		
Eunotia spp.	+	+	+	+
Fragilaria spp.	+	+	+	+
Gomphonema spp.	+	+		+
Gymnodinium spp.	+	+	+	+
Haematococcus spp.				+
Melosira granulata		+		
Melosira spp.	+			
Navicula spp.	+	+	+	+

Neidium affine	+			+
Nitzschia spp.	+	+	+	+
Oedogonium spp.		+		
Oocystis spp.				+
Pediastrum boryanum var. cornutum	+			
Pediastrum spp.	+	+	+	+
Phacus spp.	+	+		+
Pinnularia spp.	+	+	+	+
Rhoicosphenia spp.	+		+	+
Rhopalodia gibba	+		+	+
Scenedesmus spp.	+	+	+	+
Sphaerocystis spp.				+
Staurosira construens		+		
Stephanodiscus spp.	+	+		+
Stigeoclonium spp.	+			+
Synedra spp.	+	+	+	+
Synedra ulna	+	+	+	+
Synura spp.				+
Trachelomonas spp.	+	+		
Volvox spp.	+			+
Zygnema spp.	+			

Phytoplankton Per Species Density

The phytoplankton community composition varied among wetlands, with each site characterized by several dominant taxa - specifically the top 15 species per wetland (Table 4.13). In HM, the assemblage was dominated by *Trachelomonas* spp. (1.65×10^6 cells/L), *Phacus* spp. (6.38×10^5 cells/L), and *Gymnodinium* spp. (5.63×10^5 cells/L). Other abundant taxa included *Eunotia* spp., *Chlamydomonas* spp., and *Cymbella* spp., all exceeding 4.5×10^5 cells/L. LM displayed more distribution of taxa, with *Amphora* spp., *Nitzschia* spp., and *Scenedesmus* spp., each measuring approximately 3.4×10^5 cells/L. Additional species such as *Trachelomonas* spp., *Navicula* spp., and *Eunotia* spp. were also common. In LC, *Gymnodinium* spp. (8.75×10^5 cells/L) and *Eunotia* spp. (5.75×10^5 cells/L) were the dominant taxa, followed by *Navicula* spp.

and *Rhopalodia gibba* (3-5 × 10^5 cells/L). Other abundant epiphytes were identified, including *Cymbella* spp., *Fragilaria* spp., *Cocconeis* spp., and *Achnanthes* spp. VP was dominated by *Gymnodinium* spp. (9.50 × 10^5 cells/L) and *Eunotia spp.* (6.50 × 10^5 cells/L), with *Synedra ulna*, *Gomphonema* spp., and *Euglena* spp. (3-4 × 10^5 cells/L). Across all wetlands, *Gymnodinium* spp., *Eunotia* spp., *Navicula* spp., *Scenedesmus* spp., and *Cymbella* spp. were consistently present, representing the most widespread genera observed during the study.

Table 4.13 - Top 15 Phytoplankton Species present at each wetland base on PSD.

Wetland	Phyto Species	PSD (Cells/L)
Holland Marsh	*Trachelomonas* spp.	1.65E+06
Holland Marsh	*Phacus* spp.	6.38E+05
Holland Marsh	*Gymnodinium* spp.	5.63E+05
Holland Marsh	*Eunotia* spp.	5.50E+05
Holland Marsh	*Chlamydomonas* spp.	5.00E+05
Holland Marsh	*Cymbella* spp.	4.75E+05
Holland Marsh	*Scenedesmus* spp.	4.63E+05
Holland Marsh	*Nizschia* spp.	3.25E+05
Holland Marsh	*Synedra* spp.	3.25E+05
Holland Marsh	*Navicula* spp.	3.13E+05
Holland Marsh	*Gomphonema* spp.	2.50E+05
Holland Marsh	*Rhopalodia gibba*	2.00E+05
Holland Marsh	*Stephanodiscus* spp.	1.88E+05
Holland Marsh	*Achnanthes* spp.	1.63E+05
Holland Marsh	*Pediastrum* spp.	1.50E+05
Victoria Point	*Gymnodinium* spp.	9.50E+05
Victoria Point	*Eunotia* spp.	6.50E+05
Victoria Point	*Synedra ulna*	4.13E+05
Victoria Point	*Gomphonema* spp.	3.75E+05
Victoria Point	*Euglena* spp.	3.50E+05
Victoria Point	*Achnanthes* spp.	3.50E+05
Victoria Point	*Navicula* spp.	3.00E+05
Victoria Point	*Cymbella* spp.	2.63E+05
Victoria Point	*Nizschia* spp.	2.63E+05
Victoria Point	*Oocystis* spp.	2.50E+05

Victoria Point	*Scenedesmus* spp.	2.50E+05
Victoria Point	*Stigeoclonium* spp.	2.38E+05
Victoria Point	*Cocconeis* spp.	2.25E+05
Victoria Point	*Fragilaria* spp.	2.00E+05
Victoria Point	*Synedra* spp.	2.00E+05
Langman's Marsh	*Amphora* spp.	3.38E+05
Langman's Marsh	*Nizschia* spp.	3.38E+05
Langman's Marsh	*Scenedesmus* spp.	3.38E+05
Langman's Marsh	*Trachelomonas* spp.	3.00E+05
Langman's Marsh	*Navicula* spp.	2.88E+05
Langman's Marsh	*Eunotia* spp.	2.63E+05
Langman's Marsh	*Achnanthes* spp.	2.25E+05
Langman's Marsh	*Gomphonema* spp.	1.75E+05
Langman's Marsh	*Phacus* spp.	1.63E+05
Langman's Marsh	*Melosira granulate*	1.63E+05
Langman's Marsh	*Synedra* spp.	1.50E+05
Langman's Marsh	*Chlamydomonas* spp.	1.50E+05
Langman's Marsh	*Gymnodinium* spp.	1.25E+05
Langman's Marsh	*Cymbella* spp.	1.13E+05
Langman's Marsh	*Cyclotella* spp.	1.13E+05
Lagoon City	*Gymnodinium* spp.	8.75E+05
Lagoon City	*Eunotia* spp.	5.75E+05
Lagoon City	*Navicula* spp.	4.75E+05
Lagoon City	*Rhopalodia gibba*	3.00E+05
Lagoon City	*Cosmarium* spp.	2.63E+05
Lagoon City	*Cymbella* spp.	2.38E+05
Lagoon City	*Synedra* spp.	2.38E+05
Lagoon City	*Fragilaria* spp.	2.13E+05
Lagoon City	*Nizschia* spp.	1.75E+05
Lagoon City	*Rhoicosphenia* spp.	1.63E+05
Lagoon City	*Cyclotella* spp.	1.63E+05
Lagoon City	*Cocconeis* spp.	1.50E+05
Lagoon City	*Achnanthes* spp.	1.50E+05
Lagoon City	*Scenedesmus* spp.	1.38E+05
Lagoon City	*Amphora* spp.	1.25E+05

4.3.7 PCA

A PCA was used to summarize multivariate patterns in epiphyte metrics and water quality variables across wetlands. The first two components explained 69.0% of the total variance, with PC1 accounting for 46.2% and PC2 for 22.8% (Figure 4.14). PC1 showed strong positive loadings for epiphyte richness, epiphyte density, and epiphyte diversity, and strong negative loadings for water temperature, chlorophyll-*a*, and pH. PC2 was defined by strong positive loadings for TN and DOC and negative loadings for conductivity and DO.

Wetland ordinations reflected these gradients. LM scored highest on PC1, corresponding to higher epiphyte richness, density, and diversity. VP scored lowest on PC1 and highest on PC2, aligning with elevated TN and DOC. LC scored low on PC2 and moderately on PC1, while HM occupied intermediate positions on both axes.

Overall, PC1 represented the dominant chemical gradient defined by TN, DOC, conductivity, and DO. Whereas PC2 captured variation in epiphyte assemblage metrics and temperature/chlorophyll-*a* related variables (Figure 4.15).

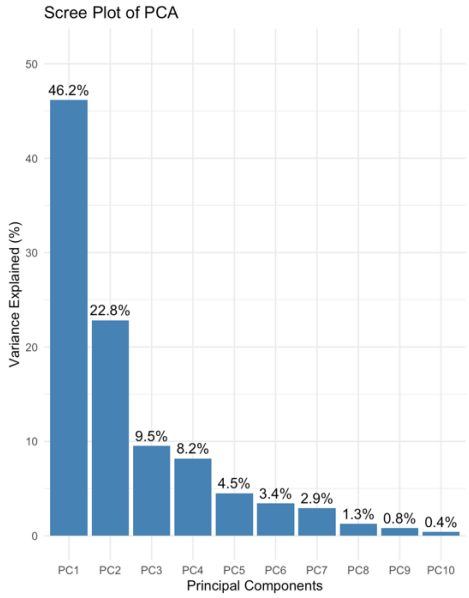
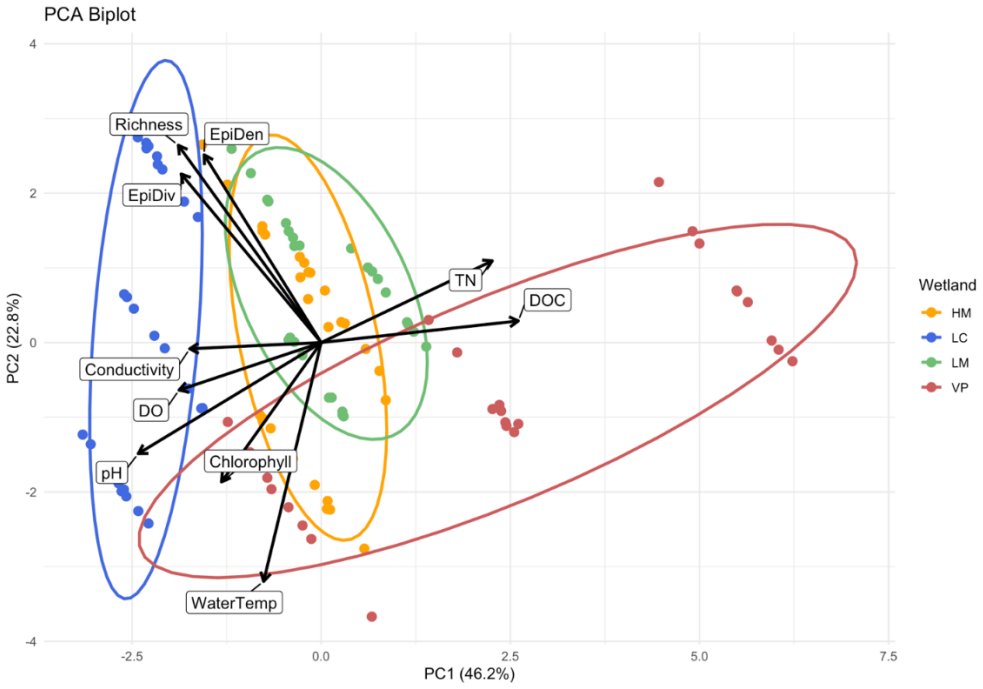


Figure 4.14 - Scree plot of principal component analysis showing the percentage of variance explained by each axis. The first two principal components accounted for 46.2% and 22.8% of the variance.



e.

Figure 4.15 - PCA biplot showing relationships between epiphyte community metrics (richness, density, diversity) and water quality variables (TN, DOC, DO, pH, conductivity, chlorophyll-a, and temperature). Ellipses illustrate distinct wetland groupings.

4.4 Discussion

4.4.1 Epiphyte Species Diversity

Seasonal patterns of average SDI calculations were consistent with previous findings in aquatic ecosystems, where epiphyte diversity typically peaks later in the growing season due to increased macrophyte surface area, greater light penetration, and enhanced resource variety. (Beck et al., 2020; Schneider et al., 2023). VP consistently exhibited significantly lower diversity than other wetlands across multiple seasons. Similar patterns have been reported in enriched or hydrologically altered wetlands, where chronic nutrient loading or turbid conditions suppress epiphytic species richness (Lange et al., 2021; Li et al., 2024).

These results align with contemporary studies showing that epiphytic algal diversity in wetlands is structured by both site-specific environmental conditions and seasonal variability. Wetlands with higher species richness and more stable diversity patterns - such as LM and LC - often display greater habitat heterogeneity, better light conditions, and more moderate nutrient regimes (Cardoso-Silva et al., 2020; Schwindt et al., 2022). In contrast, systems with persistent enrichment or physical disturbance tend to support less diverse, more opportunistic epiphytic communities.

4.4.2 Density

The analysis of epiphyte species density (cells/mm^2) across all wetlands revealed clear patterns reflecting underlying water quality conditions. Species such as *Navicula* spp. and *Nitzschia* spp., have been recognized as indicators of nutrient enrichment and organic pollution (Lange Bertalot, 1979; Van Dam et al., 1994; Lavoie et al., 2014; Porter et al., 2020). *Navicula* spp. and *Nitzschia* sp measured with the highest average densities, particularly in LC and HM, with moderately high values in LM. Their dominance could suggest nutrient pressure, although

more measurements such as diversity and richness need to be considered. In contrast, species such as *Eunotia* spp., *Synedra* spp., and *Gomphonema* spp., which are moderately sensitive to pollution and typically occur in less disturbed or lower nutrient environments (Pan et al., 1996; Stevenson et al., 2010; Bennion et al., 2018; Pandey & Lavoie, 2021), were abundant in LC and LM. This co-occurrence of tolerant and moderately sensitive taxa could point to ecological stressors starting to affect LC and LM or it could point to an overall more diverse epiphyte community. VP, which showed fewer species and more high-density taxa overall, may represent a physically constrained system as certain genera were dominating the overall abundance; the consistently low productivity warrants further investigation.

These genera specific patterns support the use of epiphyte community structure as a bioassessment tool, enabling early detection of eutrophication and guiding wetland protection and restoration strategies (Kelly et al., 1995; Resh, 2008; Cantonati et al., 2020; Feuchtmayr et al., 2019). Epiphyte density varied significantly across wetlands and seasons. This variability reflects the wide range of density values, from as low as 100 cells/mm² to more than 2,000 cells/mm² per sample. Standard deviations were high across both seasons and wetlands, indicating substantial within site variations. Seasonal trends in density were consistent with previous research supporting that epiphyte biomass and density increase later in the growing season as macrophyte degradation and nutrient release occur (Vymazal, 2013; Wetzel, 2001; Pełechata et al., 2015; Schneider et al., 2022).

The high genera specific densities observed in HM and VP align with findings from nutrient enriched or disturbed wetlands, where pollution-tolerant taxa such as *Navicula* spp., *Eunotia* spp., and/or *Nitzschia* spp. dominate overall percentages (Whitton et al., 2014; Biggs & Kilroy, 2000; Wu et al., 2017; Jiang et al., 2021). These genera are well established indicators of

eutrophic conditions and have been observed to dominate in Ontario wetlands subject to agricultural or urban runoff (Chow-Fraser, 2006; Creed et al., 2018). Conversely, VP's lower epiphyte density and reduced genera variability are comparable to oligotrophic wetlands, where stable nutrient regimes limit epiphytic biomass (Dodds & Smith, 2016; Schneider et al., 2020).

4.4.3 Species Richness

Changes in epiphyte community structure between wetlands with differing impact levels indicate that anthropogenic stressors and degraded conditions influence epiphyte assemblages, supporting the utility of epiphyte-based metrics as indicators of wetland ecological integrity (Szabó et al., 2020; Cantonati et al., 2024). The presence of both pollution tolerant taxa (e.g., *Nitzschia* spp., *Navicula* spp.) and moderately sensitive species (e.g., *Cymbella* spp., *Gomphonema* spp.) in LC suggests that intermediate nutrient conditions may promote coexistence and increased diversity, consistent with research linking diatom community shifts to nutrient enrichment gradients (Chen et al., 2025). Seasonally, richness peaked in fall across all sites, while lower spring values likely resulted from early colonization dominated by opportunistic species - a pattern reported in other temperate aquatic systems where early season assemblages are less diverse and dominated by fast-growing diatoms (Antonacci et al., 2022). These patterns support prior research showing that epiphytic richness and composition are shaped by both nutrient availability and temporal succession throughout the growing season (Guseva et al., 2025)

4.4.4 Epiphyton and Phytoplankton Comparisons

At HM, the phytoplankton community is comparatively strong: it has the highest richness (37 taxa), high diversity (SDI ≈ 2.55), and the highest mean density (~4.06×10^6 cells/L). The water column is dominated by euglenoids and dinoflagellates (*Trachelomonas* spp., *Phacus* spp.,

Gymnodinium spp.) with abundant diatoms and green algae. The epiphyte community, by contrast, is moderately rich and diverse (56 taxa total; average richness 18.63) with intermediate densities and high variability. Epiphytes are dominated by benthic diatoms such as *Navicula* spp., *Eunotia* spp., *Cocconeis* spp., *Cymbella* spp., and *Synedr*a spp., plus a handful of taxa unique to HM. Overall, compared to the other sampled wetlands, HM is more strongly expressed as a phytoplankton-rich system with midrange, variable epiphyte assemblages (Oleksy et al., 2020; Wilkinson et al., 2020; Smucker et al., 2022).

LC shows a clear contrast between compartments. The phytoplankton community is the least diverse and least rich of the wetlands (SDI 2.34; 21 taxa; density ~2.68×10^6 cells/L), with *Gymnodinium* spp. and *Eunotia* spp. dominating and fewer supporting taxa. In contrast, the epiphyte community at LC is the most developed of all sites, with the highest average richness (24.85), the highest total species count (68 taxa), and the highest epiphyte densities overall. Epiphytes are dominated by *Navicula* spp., *Eunotia* spp., *Cymbella* spp., *Achnanthes* spp., and *Rhopalodia* spp., with numerous additional genera and several species unique to LC.

Characterizing LC as having relatively simple phytoplankton communities but exceptionally rich and dense epiphyte assemblages, indicating that much of the algal structural complexity is concentrated on macrophytes rather than in the open water (Zhang et al., 2023). LC had the most hydrodynamic activity of all of the wetlands, which could result in the epiphytes having a greater advantage than phytoplankton (Gignac et al., 2022; Herstoff & Hotaling, 2023).

VP shows the opposite pattern to LC. The phytoplankton assemblage has moderate diversity and richness (SDI 2.48; 32 taxa; density ~2.73×10^6 cells/L) but is strongly dominated by a few taxa, particularly *Gymnodinium* spp. and *Eunotia* spp., with *Synedra ulna*,

Gomphonema spp., and *Euglen*a spp.. The epiphyte community, however, has the lowest average richness (12.74), the lowest total species count (50 taxa), and the lowest densities. Epiphytes are chiefly a small set of diatom genera (*Eunotia* spp., *Navicula* spp., *Nitzschia* spp., *Pinnularia* spp., *Synedra* spp.) with fewer rare or unique taxa. Overall, VP can be described as a phytoplankton-dominated wetland with simplified, low-diversity epiphyte communities, consistent with conditions where water-column blooms overshadow attached algae (Masouras et al., 2021; Rose et al., 2023). Similarly, this wetland has less hydrodynamic activity possibly creating an advantage for phytoplankton (Zhu et al., 2019).

At LM, phytoplankton and epiphytes are both relatively balanced and diverse. Phytoplankton show the highest SDI (2.61), high richness (32 taxa), and a fairly high density (~3.54×10^6 cells/L), with no single taxon overwhelmingly dominant; *Amphor*a spp., *Nitzschia* spp., *Scenedesmus* spp., *Trachelomonas* spp., and *Navicula* spp. all contribute substantially, although many other genera have healthy populations. The epiphyte community is also species rich (55 taxa; average richness 20.85) with intermediate densities and relatively even composition. Dominant epiphytes include *Navicula* spp., *Eunotia* spp., *Fragilaria* spp., *Gomphonema* spp., *Nitzschia* spp., *Synedra* spp., and several indicator taxa unique to LM. Compared with the other wetlands, LM supports well-developed, even communities in both the water column and on macrophyte surfaces, suggesting a stable, moderately productive system (Rocha et al., 2020; Cantonati et al., 2020; Trottier et al., 2022).

Across wetlands, phytoplankton fluctuated mainly by season, with densities consistently peaking in summer and dropping in spring, while diversity changed little among sites. In contrast, epiphytes fluctuated mainly by wetland, with strong spatial differences in richness, diversity, and density, and only moderate seasonal shifts (generally highest in fall). At HM, LM,

and LC, phytoplankton showed clear summer increase, whereas epiphytes showed fall peaks and greater site-specific variability (Rocha et al., 2020; Wijewardene et al., 2022). VP stood out as the only wetland where both seasonal and spatial fluctuations were dampened, with consistently low epiphyte diversity and density and comparatively stable but high levels of phytoplankton (Herstoff & Hotaling, 2023; Zhang et al., 2023). Overall, phytoplankton are season driven and relatively uniform across wetlands (Oleksy et al., 2020; Saros & Anderson, 2021), while epiphytes are wetland driven and much more sensitive to local conditions, showing stronger spatial differentiation and distinct fluctuation patterns among HM, LM, LC, and VP (Smucker et al., 2022; Masouras et al., 2021; Cantonati et al., 2020).

4.4.5 PSD Top 15 Epiphyte Species

Each wetland supported a significantly distinct epiphyte community ($p < 0.05$). Although taxa such as *Navicula* spp. and *Nitzschia* spp. occurred across all sites, their densities and dominance varied, creating unique assemblage structures. The occurrence of *Euglena* spp. and *Craticula* spp. at LC is generally associated with nutrient rich systems, as Euglena often thrives in wetlands with organic matter input but is not necessarily indicative of severe degradation in controlled populations (Szabó et al., 2020; Zhai et al., 2023). Likewise, colonial green algae such as *Eudorina spp.* are typically linked to good light penetration and nutrient availability - as their populations are not dominating, suggests that LC supports a productive yet relatively healthy algal community (Im et al., 2023). LM also showed signs of higher ecological integrity, with taxa characteristic of less disturbed habitats - a pattern consistent with studies showing shifts in phytoplankton/diatom assemblages along environmental gradients (Chen et al., 2025; Szabó et al., 2020). In contrast, species unique to HM and VP indicate greater anthropogenic stress: systems with elevated nutrients or disturbance often show dominance of opportunistic, pollution-

tolerant taxa (Im et al., 2023; Zhai et al., 2023). Taken together, the composition of site-specific taxa suggests that LC and LM represent comparatively healthier, more diverse wetlands, while VP and HM exhibit assemblages consistent with nutrient enrichment and anthropogenic influence - supporting the water quality analysis results.

Seasonal patterns were consistent across wetlands, with richness and abundance peaking in fall. This likely reflects macrophyte senescence and nutrient release, which increase substrate and nutrient availability for colonization (Szabó et al., 2020; Chen et al., 2025). Nutrient enriched wetlands such as VP exhibited more pronounced seasonal fluctuations, consistent with unstable conditions (Im et al., 2023; Zhai et al., 2023).

4.4.6 Wetland Performance Scores

The performance scoring framework applied here mirrors established bioassessment approaches that integrate biological integrity and water quality variables into a single index (Karr, 1981; U.S. EPA, 2016; Stevenson & Bahls, 1999; Environment Canada, 2004; Poikane et al., 2020; Kelly et al., 2021). This approach simplifies interpretation by translating complex datasets into scores that differentiate wetlands along a gradient of ecological condition. LC and LM reflected less impacted systems with higher representation of diverse taxa and balanced assemblages, whereas HM and VP were more nutrient enriched, dominated by tolerant taxa, and exhibited reduced richness and lower performance scores. HM displayed the highest conductivity (782 µS/cm), low chlorophyll a (1.09 mg/L), and high variability in epiphyte density, along with dominance by tolerant taxa such as *Navicula* spp. and *Nitzschia* spp. This profile reflects nutrient enriched conditions similar to degraded wetlands documented in Southern Ontario, where high conductivity and nutrient inputs drive population shifts toward tolerant genera and reduced ecological stability (Chow-Fraser et al., 1998; Bartozek et al., 2020; Winter et al., 2021). VP

scored the lowest overall, with reduced species richness (50 species), the lowest mean richness (12.74), and low density despite favorable water chemistry (lowest conductivity = 339 µS/cm; highest chlorophyll-a = 2.18 mg/L). This suggests that, while chemical conditions were suitable, biological metrics alone remained constrained (Chow-Fraser et al., 1998; Cantonati et al., 2020). Although turbidity was not measured, VP's notably dark water likely restricted light availability to epiphytes. In contrast, LM and LC demonstrated higher biological integrity and stability. LC scored highest overall, with 68 total species, mean richness 24.85, and strong DO (11.08 mg/L). LM followed with 55 species and richness 20.85, coupled with low conductivity (423 µS/cm) and near neutral pH (7.39), representing stable conditions comparable to minimally impacted Ontario wetlands (Kingsbury et al., 2012; Mackay & Robinson, 2021; Metsaranta et al., 2020). Both wetlands exhibited balanced water quality and higher biological performance, consistent with patterns linking lower nutrient levels to greater diversity and stability (Ontario Ministry of the Environment, 2016; Dodds & Smith, 2021). These results confirm an ecological gradient, with HM and VP showing characteristics of more impacted, nutrient enriched or biologically limited systems, while LM and LC represent less impacted, more stable wetlands with stronger biological diversity and balanced water quality.

4.4.7 PCA

The PCA results highlight clear relationships between epiphyte community structure and environmental variables across the four wetlands. Epiphytic algae responded predictably to gradients in water quality, particularly nutrient loading, oxygen availability, and conductivity (Smucker et al., 2022; Yuan et al., 2024). LM displayed the most favorable conditions for epiphyte diversity and density, clustering with high scores for richness, density, and diversity. LC was associated primarily with higher pH, DO, and conductivity, representing ion rich,

oxygenated waters. In contrast, VP was strongly associated with elevated TN and DOC, indicative of nutrient enrichment and organic loading. These conditions are characteristic of eutrophic systems that often experience algal blooms, light limitation, and epiphyte population shifts toward more pollution tolerant species (Taylor et al., 2006; Stevenson et al., 2008; Suresh et al., 2023; Brehob et al., 2024). VP was clearly separated from the other wetlands along the positive PC1 axis, reflecting its distinct ecological status. Correspondingly, VP exhibited the lowest values for epiphyte richness, diversity, and density, suggesting that excessive nutrient and organic inputs reduce diversity and cause loss of sensitive taxa. These PCA patterns are consistent with the broader algal dynamics observed across the wetlands, where sites with higher phytoplankton biomass - particularly VP - also showed the lowest epiphyte richness and density, underscoring the reciprocal relationship between water column blooms and the suppression of attached algal communities.

HM, located centrally in the ordination, represents an intermediate ecological state. It is not strongly associated with the enriched conditions of VP or the more favorable parameters of LC and LM, suggesting moderate nutrient inputs and variable water quality, possibly due to agricultural runoff. Agricultural inputs often cause fluctuating algal assemblages as nutrient pulses trigger seasonal community shifts (Chambers et al., 2006; Lavoie & Campeau, 2010; McDowell et al., 2020; Pearce et al., 2021; Trottier et al., 2022). Transitional sites such as HM may be ecologically unstable and at risk of further degradation without mitigation. The negative association of chlorophyll-*a* and water temperature with epiphyte richness and diversity suggests a broader stress gradient. Elevated chlorophyll-*a* indicates phytoplankton blooms that increase competition for light and nutrients, while higher temperatures can exacerbate oxygen depletion and favor fast growing, less diverse populations (Smol & Stoermer, 2010; Oleksy et al., 2020;

Rose et al., 2023). In summary, the PCA demonstrates that LC and LM support diverse, balanced epiphyte communities under favorable physicochemical conditions, whereas VP shows clear signs of biological degradation. HM occupies a transitional position, potentially reflecting episodic anthropogenic influence. These patterns are consistent with findings across Ontario and temperate North America, supporting the value of algal indicators for wetland monitoring and management (Reavie et al., 2000; Lavoie et al., 2008; Simmatis et al., 2020; Hoskin et al., 2024; Environment and Climate Change Canada & U.S. EPA, 2022; Masouras et al., 2021).

4.5 Conclusion

Data collected from HM, VP, LC, and LM revealed clear differences in epiphyte community structure and water quality, defining an ecological gradient from less impacted to more impacted wetlands. HM exhibited the highest conductivity (782 µS/cm), lowest chlorophyll-*a*, greatest DO variability, and high-density variation, coupled with dominance by nutrient tolerant taxa such as *Navicula* spp. and *Nitzschia* spp., indicating enrichment and instability. LC supported the highest diversity (68 species) and richness (24.85 species per sample), along with balanced water chemistry (high DO = 11.08 mg/L, moderate conductivity). LM displayed similarly stable conditions, with intermediate richness (55 species), near neutral pH (7.39), and low conductivity (423 µS/cm), suggesting a well functioning wetland with moderate nutrient influence. VP scored lowest overall, with reduced richness (50 species) and density, indicating potential constraints from habitat structure, hydrological isolation, or chemical stress. The alignment between epiphyte assemblages and water quality variables underscores the value of epiphytic algae as bioindicators of wetland condition. HM's nutrient driven stress was evident in its high conductivity and dominance of tolerant species, while LC and LM supported diverse communities including moderately sensitive taxa such as *Eunotia* spp.

and *Synedra* spp., reflecting ecological stability. These findings illustrate a clear gradient: LC represents a high functioning reference wetland, LM reflects stable intermediate conditions, and HM and VP show nutrient-enrichment impacts. Integrating epiphyte based bioassessment with water chemistry analysis provides a robust framework for assessing wetland health. This approach supports protection of LC, maintenance and enhancement of LM's balanced condition, nutrient-mitigation strategies and habitat restoration priorities for both HM and VP, thereby strengthening wetland management across the Lake Simcoe watershed.

Chapter 5 : The Influence of Macrophyte Hosts on Epiphyte Community Structure

5.1 Introduction

Macrophytes are plants that grow in or near aquatic environments and are foundational to wetland structure and function. They include a variety of growth forms such as submerged, emergent, and floating species, and include taxa such as *Typha* spp., *Nymphaea* spp., *Lemna* spp., etc. (Zhang et al., 2023; Wang et al., 2023). These plants increase the diversity in their habitats; they contribute to nutrient cycling and serve as hosts for diverse epiphytic communities. This host-epiphyte relationship is influenced by both physical and chemical characteristics of the macrophyte surface, and varies widely depending on species, environmental conditions, and seasonal dynamics (Awo & Fonge, 2024; Wang et al., 2024). The relationship between macrophytes and epiphytes is especially complex and ecologically significant and needs to be studied further.

Macrophyte hosts can have significant influence on the composition and productivity of their surrounding epiphytic communities. For example, certain macrophyte species may affect epiphyte populations by altering the surrounding water column, changing light availability through canopy shading, or reducing nutrient concentrations (Jaschinski et al., 2024). Some macrophytes can produce allelopathic compounds that inhibit algal photosynthesis, limiting phytoplankton and epiphytic growth, and altering their community structures (Li et al., 2022; Tóth, 2025).

Epiphytes can affect their macrophyte hosts both positively and negatively. When their populations are in check, epiphytes may provide protective benefits by absorbing harmful ultraviolet radiation or deterring herbivores (Zhang et al., 2023; Jaschinski et al., 2024). However, excessive epiphytic growth can create eutrophic conditions, reduce light penetration to

submerged macrophytes- reducing photosynthesis and ultimately limiting macrophyte growth (Awo & Fonge, 2024; Wang et al., 2024). The balance of this relationship is influenced by a variety of factors, including species identity, biomass, seasonal growth cycles, and external environmental stressors.

Significant knowledge gaps remain regarding how different macrophyte species may affect the structure of epiphyte communities under varying environmental conditions. Species-specific traits such as surface texture, tissue age, and vertical positioning in the water column may all influence epiphyte colonization and community structure (Li et al., 2022; Wang et al., 2024). Understanding these host driven patterns is especially important in wetland biomonitoring, where macrophyte composition and abundance can shift dramatically with seasonal changes or external pressures.

This chapter investigates how different macrophyte hosts influence the structure of epiphyte communities across wetlands in the Lake Simcoe watershed. The study focuses on comparing epiphyte density, richness, and diversity on multiple host species, considering how biological traits such as their location in the water column, shape, and quality of structure, as well as locations and seasonal factors, interact to shape community composition. By analyzing host specific effects on epiphyte communities, this research contributes to a deeper understanding of biotic interactions in freshwater systems and informs the use of epiphytes as sensitive indicators of wetland health.

Three types of macrophyte were used in this study to examine epiphyte community structure compared to their hosts: *Nymphaea odorata, Typha angustifolia,* and *dead Typha angustifolia*. Collectively, these three substrate types represent contrasting macrophyte forms that provide an ecologically meaningful basis for evaluating variation in epiphyte density,

diversity, and richness compared to their differing host types (emergent vs. floating) as well as host quality (dead or alive). Ultimately providing more understanding of the macrophyte role in the use of epiphytes as wetland bioindicators.

5.2 Methods

The description of methods of collection of macrophytes and extraction of epiphytes was given in Chapter 2 (Materials and Methods). The results of the measurements and sample analyses are described below.

Statistical analysis:

Statistical analyses were conducted to assess variations among wetlands and macrophyte types. Differences in macrophyte size were evaluated using a two-way ANOVA to determine the effects of wetland and macrophyte type on average leaf area. Epiphyte community structure was examined through multiple analytical approaches. A one-way ANOVA was used to compare the Shannon Diversity Index (SDI) and the species richness between macrophyte types.

A two-way ANOVA, followed by Tukey's post hoc tests, was used to identify significant variation and specific pairwise differences in epiphyte density. A nonparametric Kruskal-Wallis and Dunn's tests confirmed these patterns. A Principal Components Analysis (PCA) was then performed to visualize relationships among richness, diversity, and density.

5.3 Results

5.3.1 Epiphyte Diversity per Macrophyte

Average Shannon Diversity Index values (SDI) of epiphytes were similar among the three macrophyte types (Table 5.1). The highest average SDI occurred on TAD (mean = 2.54, SD = 0.10), followed by NO (mean = 2.43, SD = 0.14), and TA (mean = 2.37, SD = 0.17). Variability in diversity was lowest on TAD and highest on TA, but overall SDI values were

consistent across macrophytes. A one-way ANOVA detected no significant differences in mean SDI among the sampled macrophytes ($p > 0.05$).

When examined between each macrophyte type, the SDI values varied only modestly across seasons (Figure 5.1). For NO, mean SDI values ranged from 2.29 in summer to 2.43 in fall, while TA ranged from 2.27 in spring to 2.36 in fall. TAD exhibited the highest overall SDI across seasons, increasing from 2.32 in summer to 2.48 in fall. However, the Tukey post-hoc comparisons showed no significant differences in SDI across the seasons for any of the macrophytes ($p > 0.05$ as indicated by identical letter groupings across seasons within each species panel, Figure 5.1). These results show that epiphyte diversity was stable through the growing season regardless of host macrophyte.

In contrast to the seasonal patterns, SDI varied significantly across sites for each macrophyte ($p<0.05$) (Figure 5.2). For NO, the SDI values were significantly lower at VP (mean = 1.90) compared to SDI values at HM, LC, and LM (means ranging from 2.33-2.47). For TA, SDI was again lowest at VP (mean = 1.88), significantly different from HM, LC, and LM (means 2.32-2.43; Tukey $p < 0.05$). For TAD, VP also had the lowest SDI (mean = 2.10), significantly lower than LC (mean = 2.55) and LM (mean = 2.47), with HM intermediate. Across all macrophytes, VP consistently supported the lowest epiphyte diversity, while LC and LM supported the highest epiphyte diversity (the Tukey test letter groupings in Figure 5.4 highlight these patterns, with VP forming its own distinct group (“a”) for all macrophytes, while LC and LM cluster as significantly higher-diversity sites (“b”)). Together, these patterns indicate that although macrophyte identity and season had little influence on epiphyte SDI, wetland site strongly affected epiphyte diversity on each macrophyte, with VP consistently diverging from the other wetlands.

Table 5.1 - Average Shannon diversity index and standard deviation of epiphytes on Nymphaea odorata, Typha angustifolia, and dead Typha angustifolia.

Macrophyte	Average Shannon Diversity Index	Standard Deviation
Nymphaea odorata	2.43	0.14
Typha angustifolia	2.37	0.17
Typha angustifolia Dead	2.54	0.10

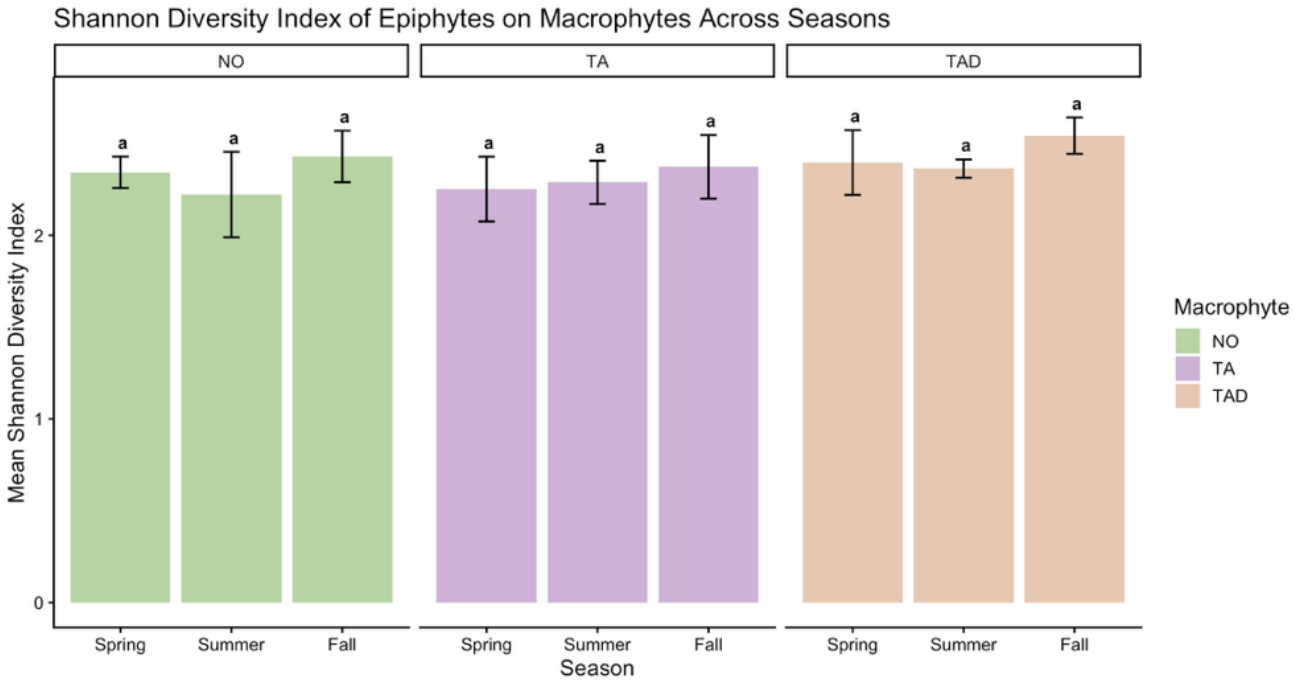


Figure 5.1 - Seasonal variation in Shannon diversity index of epiphytic algae on three macrophyte species (NO, TA, TAD). Error bars represent SD. Letters represent non-significant differences among seasons ($p > 0.05$).

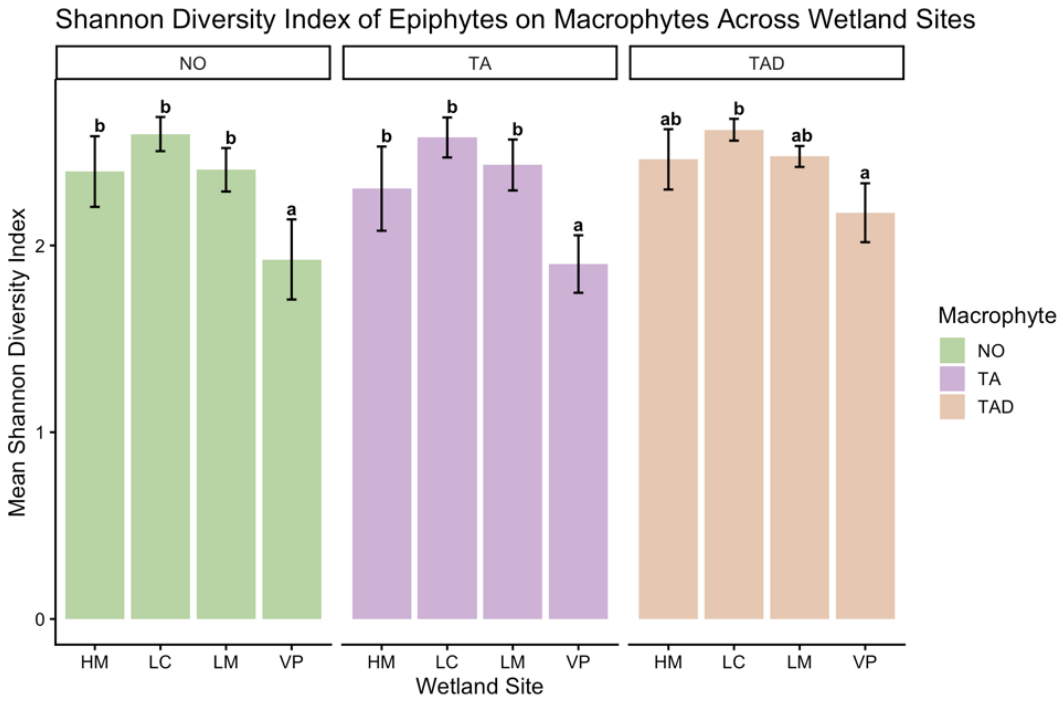


Figure 5.2 - Mean Shannon diversity index of epiphytes on the three macrophyte species across four wetland sites. Error bars represent SD. Letters denote significant differences among sites within each macrophyte.

5.3.2 Epiphyte Density Per Macrophyte

Epiphyte density differed significantly among macrophytes ($p<0.05$). Mean densities were highest on TAD, followed by NO, and lowest on TA. Tukey post-hoc tests showed that densities on TAD were significantly higher than those on both NO and TA ($p < 0.05$), while NO and TA did not differ significantly from one another in any season ($p > 0.05$) (Table 5.2).

Seasonal comparisons within macrophytes showed that NO had significantly higher epiphyte densities in fall compared to spring ($p <0.05$). TA showed no significant differences among seasons ($p > 0.05$), while TAD exhibited fall densities that were significantly higher than both spring and summer ($p<0.05$). Wetland sites had a significant effect on epiphyte density ($p < 0.05$). Across all macrophytes, VP consistently had the lowest densities, while LC and LM had the highest. Post-hoc comparisons revealed distinct patterns within macrophytes. For NO,

densities at HM, LC, and LM were all higher than at VP. For TA, densities at LC were higher than at VP ($p < 0.05$). For TAD, VP had significantly lower densities than HM, LC, and LM ($p < 0.05$). (Figure 5.3, Figure 5.4).

Site specific macrophyte comparisons further showed that at HM, LC, and LM, densities on TAD were significantly higher than on both NO and TA ($p < 0.05$). At VP, densities were uniformly low, although TAD remained higher than NO and TA. At LM and LC, significant differences were found for TAD compared to both NO and TA ($p<0.05$) (Figure 5.3, Figure 5.4).

Table 5.2 - Average density of epiphytes (/mm²) across macrophyte hosts. TAD supported the highest average epiphyte density, followed by TA and NO.

Macrophyte	Average Density of Epiphytes
Nymphaea odorata	467.55/mm^2
Typha angustifolia	614.75/mm^2
Typha angustifolia Dead	1078.01/mm^2

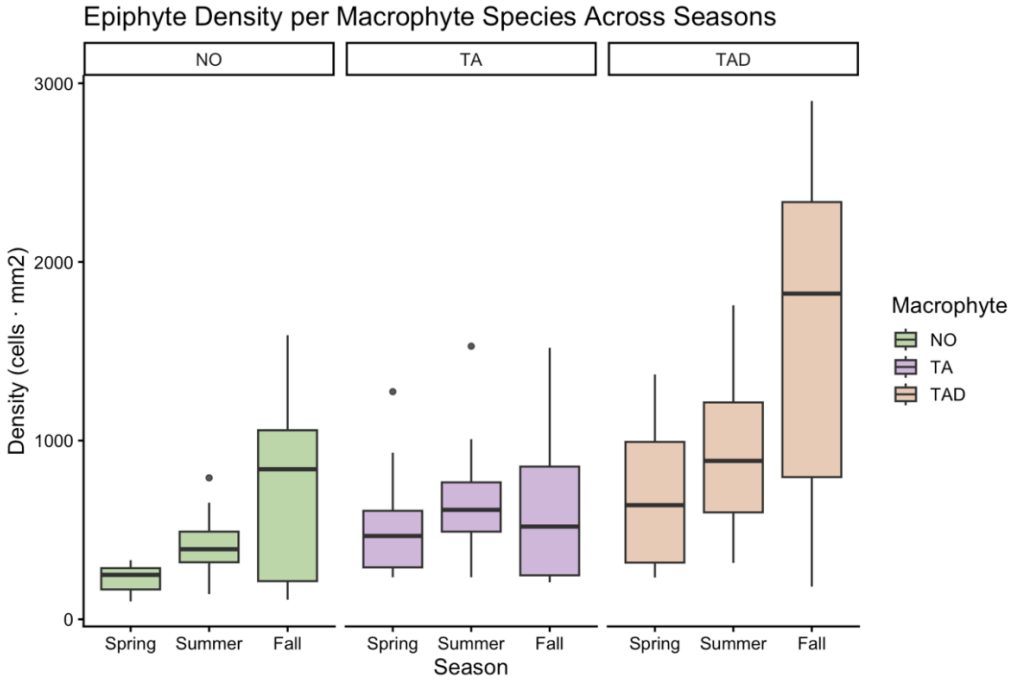


Figure 5.3 - Epiphyte density (cells/mm²) on NO, TA, and TAD macrophytes across spring, summer, and fall. Boxplots show median, interquartile range, and outliers.

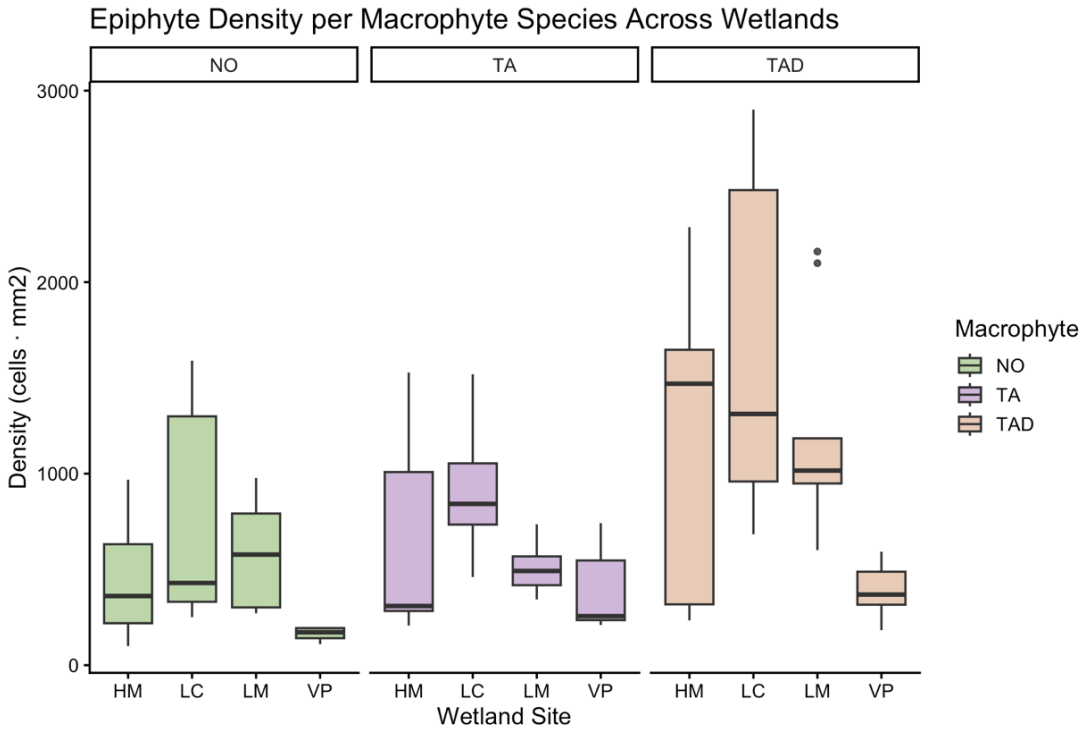


Figure 5.4 - Epiphyte density (cells/mm²) on NO, TA, and TAD macrophytes across the four wetland sites.

5.3.3 Epiphyte Species Richness Per Macrophyte

Average epiphyte species richness was similar among the three macrophytes. Mean richness ranged from 17.67 species on TA, to 21.19 species on TAD, with NO sitting in the middle with an average of 19.36 species (Table 5.3). An ANOVA detected no significant overall differences in species richness among macrophyte types ($p > 0.05$).

A two-way ANOVA testing the combined effects of macrophyte and season revealed a strong seasonal effect on richness ($p < 0.05$), while macrophyte type remained non-significant ($p > 0.05$). Post hoc comparisons showed clear but host-specific seasonal patterns. For NO, richness in the fall was significantly higher than in spring ($p < 0.05$), while differences between fall and summer and between spring and summer were not significant. A similar pattern occurred for TAD, with fall richness significantly exceeding spring ($p < 0.05$), and the fall-summer contrast

approaching but not reaching significance ($p > 0.05$). TA showed no significant differences among seasons.

Richness across wetlands showed a strong site effect ($p < 0.05$) (Figure 5.5). Post hoc comparisons within wetlands revealed that richness was consistently lowest at VP across all three macrophytes. For NO, richness at VP was significantly lower than at HM, LC, and LM ($p < 0.05$). For TA and TAD, richness at LC was significantly higher than at VP ($p < 0.05$), while differences among HM, LC, and LM were generally smaller and often non-significant.

Analysis of macrophytes within each season found no significant differences among NO, TA, and TAD during the spring or the summer ($p > 0.05$) (Figure 5.6). In the fall, epiphyte richness on TAD was significantly higher than on TA ($p < 0.05$). Differences between NO and TAD, as well as TA and NO, were non-significant ($p > 0.05$).

Table 5.3 - Average epiphyte species richness across macrophyte hosts.

Macrophyte	Average Epiphyte Species Richness
Nymphaea odorata	19.36
Typha angustifolia	17.67
Typha angustifolia Dead	21.19

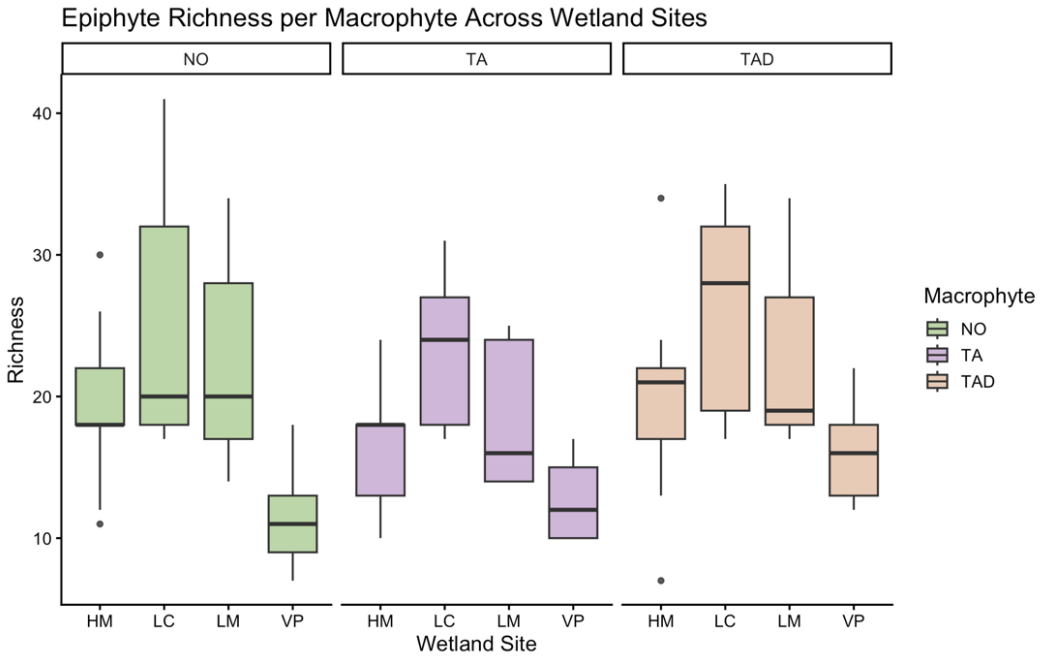


Figure 5.5 - A boxplot displaying the epiphyte richness on NO, TA, and TAD macrophytes across four wetland sites (HM, LC, LM, VP).

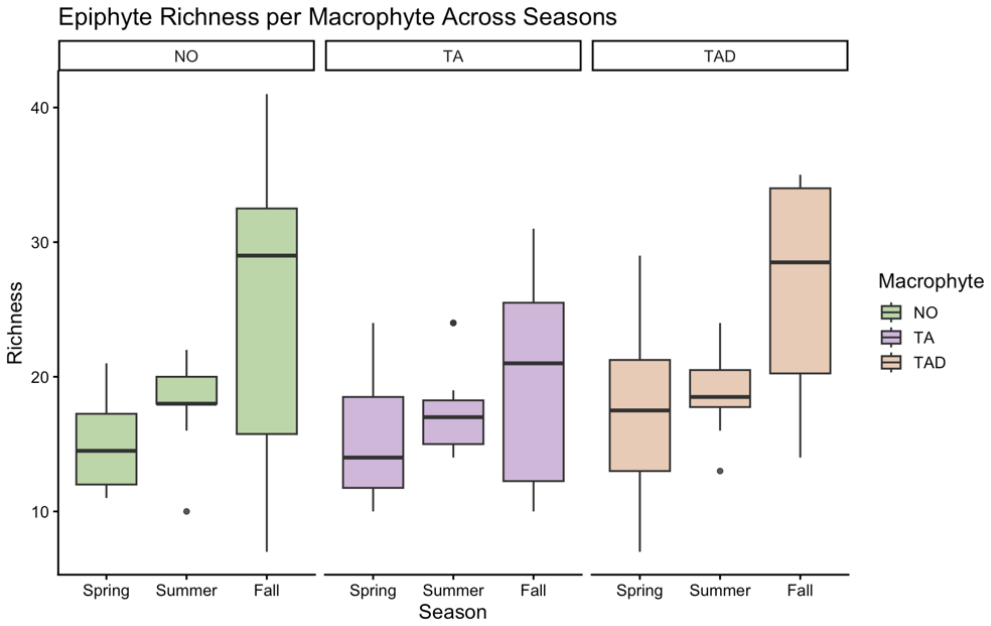


Figure 5.6 - A boxplot displaying epiphyte richness on NO, TA, and TAD macrophytes across spring, summer, and fall.

5.3.4 Epiphyte Per Species Density (PSD) on Per-Species Macrophyte.

To determine whether PSD accumulation differed among the three macrophyte species, a one-way ANOVA was conducted. The analysis showed a significant difference of PSD between macrophyte species ($p<0.05$). A Tukey post-hoc tests revealed that TAD had significantly higher PSD than both NO and TA ($p<0.05$). NO and TA did not differ significantly from each other ($p>0.05$).

Across all macrophytes, seasonal patterns in PSD accumulation were species specific. NO showed a significant seasonal effect, with total PSD measuring the highest in the Fall and the lowest in the Spring ($p<0.05$). TA showed no significant variation in PSD across either season or wetland, indicating relatively stable PSD accumulation regardless of environmental conditions ($p>0.05$). TAD also showed no significant seasonal or wetland effects ($p>0.05$). Overall, seasons only had an effect on PSD variation for NO, whereas wetland had no significant influence on PSD accumulation for any macrophyte ($p>0.05$).

Analysis of PSD patterns showed clear differences in dominant epiphyte genera among the macrophyte hosts (Table 5.4; Figures 5.7-5.8). Across all wetlands and seasons, NO samples were most strongly characterized by higher densities of *Navicula* spp. and *Eunotia* spp., followed by *Synedra* spp., *Cymbella* spp., and *Eunotia fennica*. TA macrophytes exhibited a similar species profile, with *Navicula* spp. and *Eunotia* spp. as the most abundant taxa, but they were followed by elevated densities of *Nitzschia* spp., *Synedra* spp., and *Rhopalodia gibba*. TAD hosts supported the highest overall per-species densities, also dominated by *Navicula* spp. and *Eunotia* spp., with consistently high PSD from *Synedra* spp., *Cymbella* spp., and *Achnanthes* spp.

Table 5.4 - Top 15 most abundant epiphyte species per macrophyte, in order.

Nymphaea odorata	*Typha angustifolia*	*Typha angustifolia - dead*
Navicula spp.	*Navicula spp.*	*Navicula spp.*
Eunotia spp.	*Eunotia spp.*	*Eunotia spp.*
Synedra spp.	*Nitzschia spp.*	*Synedra spp.*
Cymbella spp.	*Synedra spp.*	*Cymbella spp.*
Eunotia fennica	*Rhopalodia gibba*	*Achnanthes spp.*
Gomphonema spp.	*Cymbella spp.*	*Nitzschia spp.*
Achnanthes spp.	*Gomphonema spp.*	*Rhoicosphenia spp.*
Fragilaria spp.	*Rhoicosphenia spp.*	*Rhopalodia gibba*
Nitzschia spp.	*Cocconeis spp.*	*Gomphonema spp.*
Cocconeis spp.	*Scenedesmus spp.*	*Fragilaria spp.*
Scenedesmus spp.	*Bulbochaete spp.*	*Bulbochaete spp.*
Rhoicosphenia spp.	*Fragilaria spp.*	*Cocconeis spp.*
Synedra ulna	*Achnanthes spp.*	*Scenedesmus spp.*
Oocystis spp.	*Stephanodiscus spp.*	*Synedra ulna*
Bulbochaete spp.	*Diatoma spp.*	*Eunotia fennica*

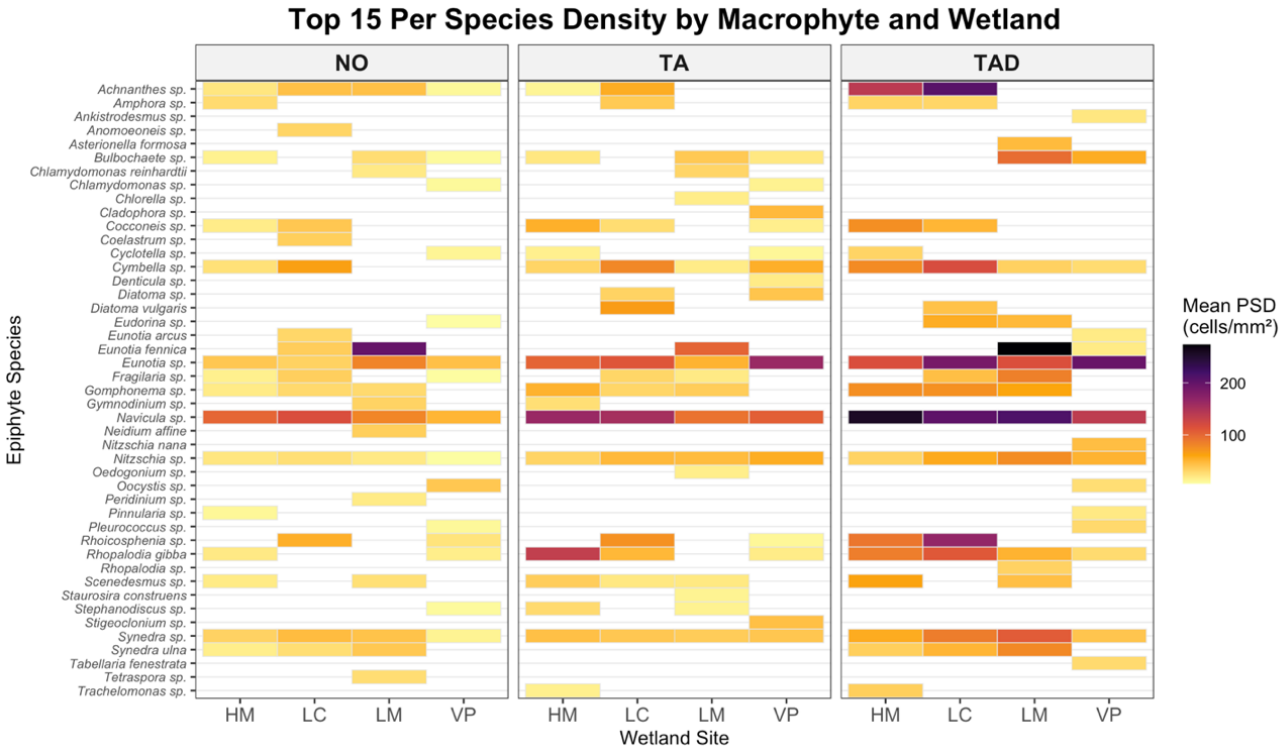


Figure 5.7 - Heatmap showing the top 15 epiphyte species ranked by per-genera density across wetlands for each macrophyte. Darker colors represent higher mean PSD values, illustrating differences in dominant genera among macrophytes and across sites.

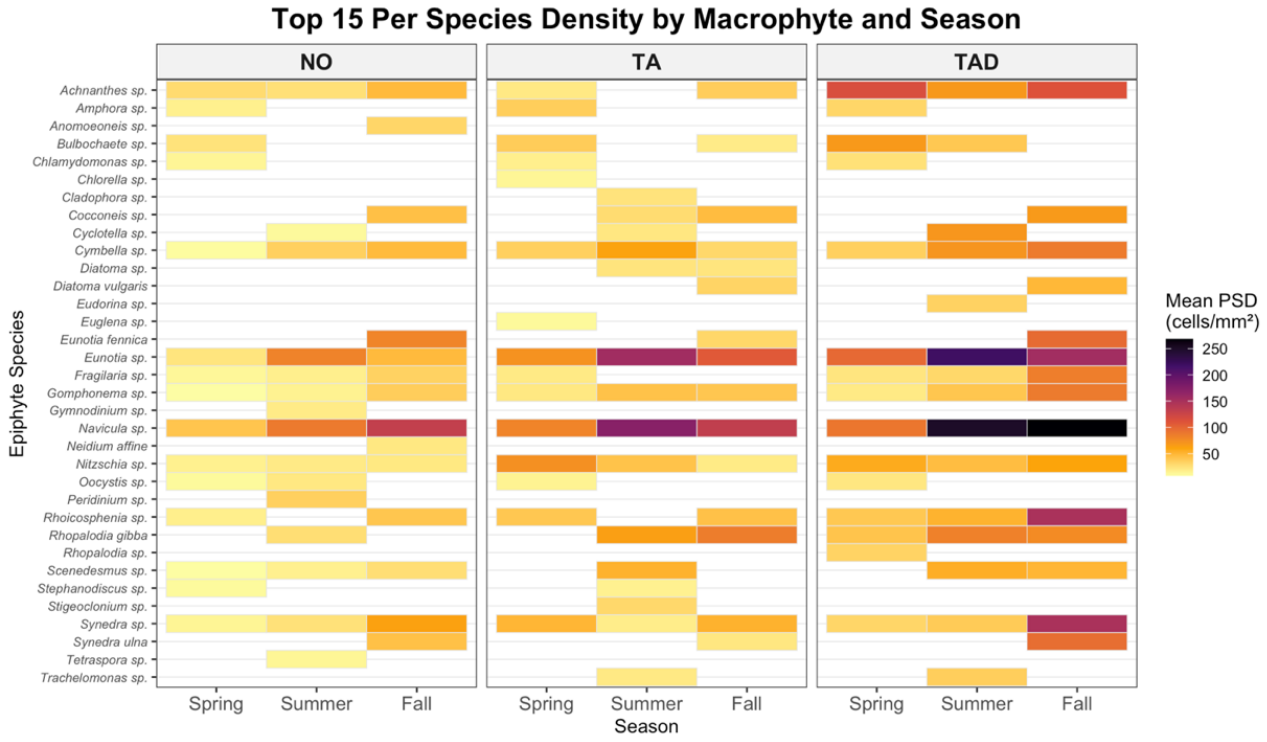


Figure 5.8 - Heatmap showing the top 15 epiphyte species per macrophyte, based on mean per-genera density across seasons. Darker colors indicate higher PSD values, highlighting seasonal shifts in dominant taxa within each macrophyte group.

5.3.5 Principal Component Analysis

A principal component analysis was used to explain variation in epiphyte richness, diversity, and density across macrophytes and seasons. The first two components explained 94.8% of the total variance, with PC1 accounting for 81.5% and PC2 explaining 13.3% (Figure 5.9). PC1 represented the dominant gradient in overall epiphyte magnitude. All three epiphyte metrics loaded strongly and negatively on PC1.

PC1 differentiates samples where the epiphyte assemblage tends to have higher diversity but lower density from those where the assemblage exhibits higher density but lower diversity. Because the loadings are relatively small, this gradient is weaker than the primary one captured by PC2, but it still reflects a secondary pattern in community composition.

TAD samples tended to plot further left on PC2, corresponding to higher overall richness, diversity, and density. In contrast, NO samples were generally located to the right and slightly higher on PC1, indicating lower overall epiphyte values and a subtle shift toward proportionally higher diversity. TA samples occupied intermediate positions along both axes, consistent with moderate epiphyte levels. Seasonal patterns were comparatively weak. Fall, spring, and summer samples were interspersed throughout the ordination, suggesting that seasonal effect had much less influence on epiphyte community structure than macrophyte type (Figure 5.10).

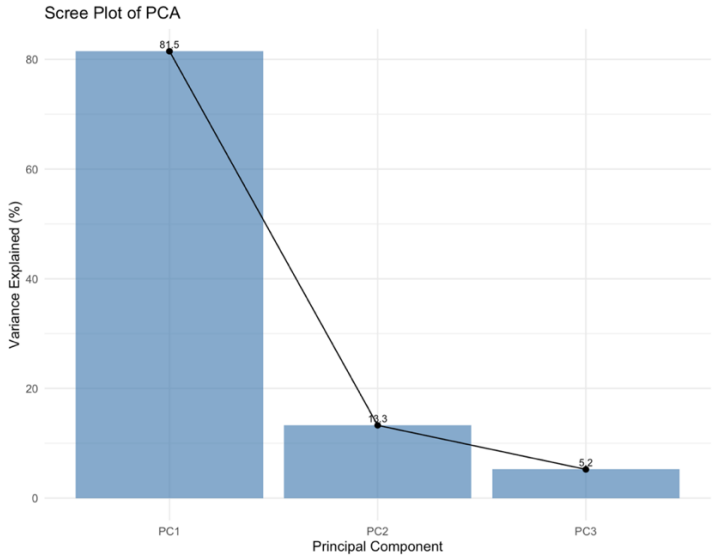


Figure 5.9 - Scree plot of PCA showing that PC1 accounted for the majority of the variation in epiphyte community structure (81.5%), followed by PC2 (13.3%), indicating that most variation was explained by the first two components.

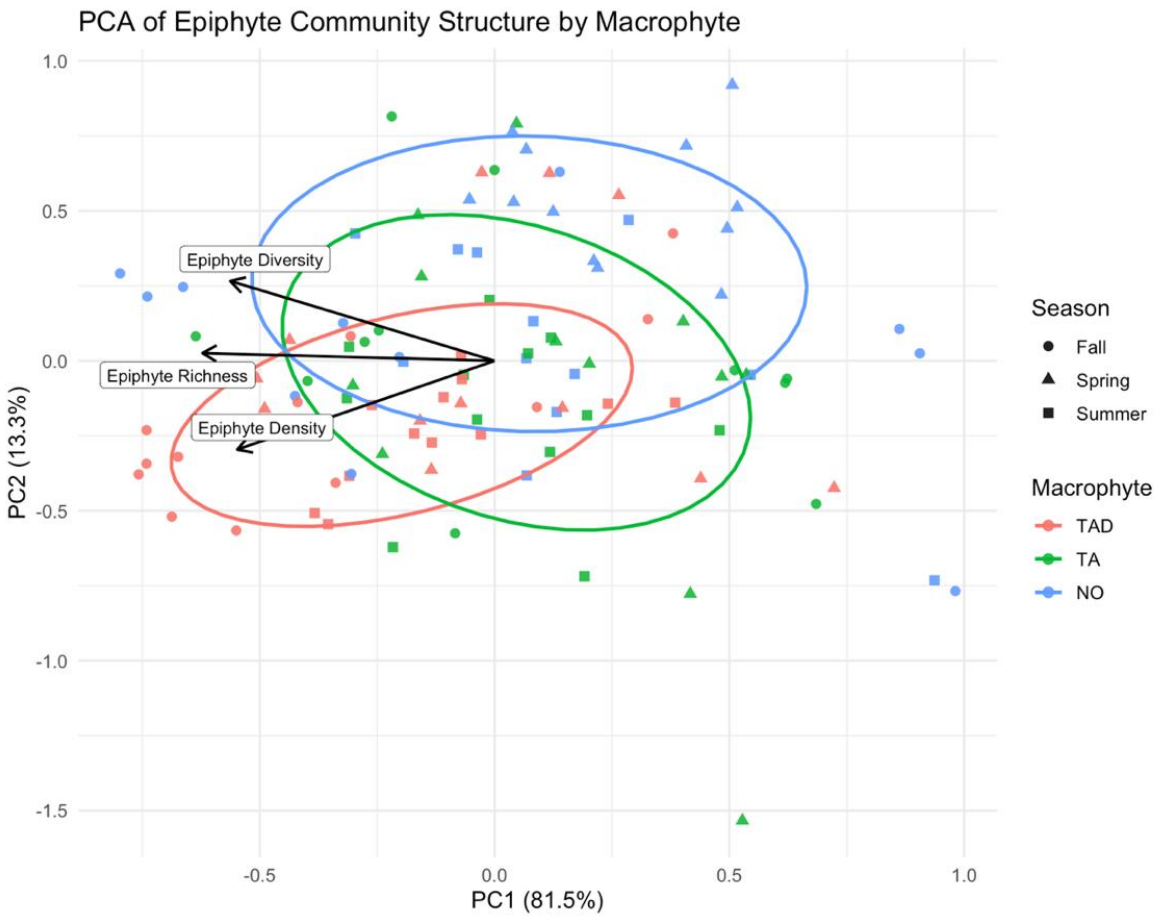


Figure 5.10 - PCA of Epiphyte richness, diversity, and density across macrophyte hosts (TAD, TA, NO) and seasons. PC1 (81.5%) reflects a gradient contrasting higher diversity with higher density (negative). PC2 (13.3%) captures the gradient in epiphyte magnitude.

5.4 Discussion

5.4.1 Epiphyte Diversity Per Macrophyte

Epiphyte diversity showed no significant differences with macrophyte hosts, indicating that host identity does not have a role in shaping the diversity of epiphytic assemblages in these wetlands. This aligns closely with a recent study showing that epiphyte diversity in temperate freshwater systems is more often determined by environmental gradients rather than host-specific traits (Bae & Liess, 2020; Tapia-Grimaldo et al., 2021; Grasset, 2023).Seasonal stability in SDI across all macrophytes further supports the interpretation that short-term temporal

changes have relatively little influence on epiphyte diversity, as long as the baseline environmental conditions remain within the tolerance range. This consistent seasonal pattern agrees with earlier studies showing that the diversity fluctuation is less than that of biomass, richness, or per-species density, because many epiphyte taxa have wide environmental tolerances (Bellinger & Cocquyt, 2022).The diversity patterns observed here reinforce the conclusion that wetland environmental conditions, not macrophyte species, are the dominant filters controlling epiphyte diversity in these systems.

5.4.2 Epiphyte Density Per Macrophyte

Epiphyte density varied strongly among macrophyte species and among wetlands, indicating that biomass accumulation is governed by both macrophyte condition and wetland water quality. Across all sites, TAD supported the highest density, consistent with the recent research showing that senescent macrophytes promote increased periphyton biomass due to structural and biochemical changes occurring during the decay process. As the tissue dies, they exhibit reduced chemical defenses, increased surface roughness, sediment trapping, and slower tissue turnover, all of which can control epiphyte settlement (Rojo et al., 2021; Sundbäck et al., 2020; Reavie & Kireta, 2023). Studies in freshwater and wetland ecosystems found that decaying substrates accumulate significantly more epiphytes than living tissues (Cano et al., 2013; Wijewardene et al., 2022).

In contrast, living NO and TA hosted lower densities, reflecting active mechanical and chemical defense traits. NO possesses a hydrophobic, wax-coated cuticle that reduces algal adhesion and slows initial colonization (Chambers et al., 2021). TA’s vertical orientation, exposure to wind movement stress, and more rigid leaf architecture reduce the stability of

attached biofilms (Carpenter et al., 2020). These traits help explain the consistent ranking of densities across wetlands-TAD the highest, and no significant difference between NO and TA.

Seasonal patterns generally followed expectations. NO and TAD exhibited elevated densities in the fall, aligning with well documented fall nutrient pulses that increase algae biomass in wetland systems (Rocha et al., 2020). TA did not exhibit a fall surge in epiphyte density. The average TA densities were more tightly clustered across seasons, indicating a lower temporal variability. This may reflect its production of allelopathic compounds shown to inhibit algal and cyanobacterial growth (Kang et al., 2020), thereby dampening nutrient-driven biomass increases. Taken together, these results support a two-level mechanism controlling epiphyte density on macrophytes: (1) Substrate condition (living vs. senescent) determines the potential for colonization, while (2) Wetland level nutrient and hydrological dynamics determine how fully that potential is realized.

5.4.3 Epiphyte Species Richness

Epiphyte species richness showed no significant difference among macrophytes, indicating that macrophyte species and type only had a limited influence on species richness compared to environmental and seasonal factors. Epiphyte communities are known for their persistence across substrates, contributing to relatively stable richness regardless of host species (Vadeboncoeur et al., 2021; Grasset, 2023).

Richness differences among macrophytes within individual seasons were mostly non-significant, further reinforcing that richness is controlled more by regional nutrient/light regimes, DOC levels, etc., than by properties of the host plant itself (Liu et al., 2022; Vadeboncoeur et al., 2021). Only in the fall did TAD show significantly higher richness than TA, consistent with the idea that senescent substrates provide more ecological niches than living emergent tissues. TA's

lack of seasonal increase is notable. Even though densities change moderately in TA in some seasons, its species richness remains comparatively constrained. Considering TA is known to release allelopathic compounds that suppress certain algal taxa (Kang et al., 2020), this may also reduce colonization by more sensitive species, dampening richness shifts across seasons.

Richness differed significantly among wetlands, indicating that site-level environmental regimes were the dominant driver of epiphyte species diversity. Across macrophytes, LC consistently supported the highest richness, while VP consistently supported the lowest, with significant pairwise differences between VP and the other sites for NO, and between VP and LC for TA and TAD. Lower richness at VP suggests that local environmental conditions constrain both the number of taxa and their ability to establish persistent populations. This pattern is broadly consistent with studies showing that site-specific light, nutrient, etc. can strongly modulate epiphyte richness and composition (Teittinen et al., 2021; Gignac et al., 2022; Somma et al., 2023).

LC and LM supported both higher richness and higher epiphyte densities relative to VP. This pattern suggests that more favorable nutrient and light conditions at these wetlands promote coexistence of multiple taxa, rather than strong competitive dominance by a few species. Elevated nutrient availability and relatively stable hydrology can increase biomass while maintaining or slightly enhancing richness, depending on the balance between resource supply and competitive interactions (Somma et al., 2023; Werner et al., 2013).

These findings align with a growing consensus that epiphyte species richness is governed primarily by environmental conditions rather than host identity. However, macrophyte type still exerted a measurable influence on richness in specific contexts (e.g., senescent tissue), indicating that host characteristics should not be ignored. Although richness remained relatively stable

across substrates, subtle but consistent host-specific effects suggest that macrophyte identity warrants consideration in study design and interpretation (Vadeboncoeur et al., 2021; Grasset, 2023; Liu et al., 2022).

5.4.4 Per Species Density per Macrophyte

PSD analyses revealed macrophyte driven differences in epiphyte assemblages, with TAD consistently supporting significantly higher PSD values than both NO and TA. This pattern highlights the importance of substrate condition, tissue integrity, and chemical status in structuring. The high PSD on TAD reflects the previously discussed changes from living to senesce macrophyte tissue. As stated earlier, when *Typha* spp. leaves die, they undergo changes that make them more ideal for epiphyte population growth. In contrast, NO and TA supported lower and more compositionally constrained PSD assemblages. Possibly due to the same structure observations with NO's wax-rich leaf surfaces and alive *Typha's* allelopathic effects (Chambers et al., 2021; Gross, 2023). These mechanisms likely restrict the proliferation of dominant taxa, resulting in a comparatively lower PSD range.

Seasonally, PSD for NO was highest in the fall, reflecting autumn nutrient regeneration, cooler temperatures, and reduced grazing. TA and TAD exhibited no significant seasonal PSD patterns and remained consistent throughout the year. Overall, PSD results reveal that while epiphyte richness and diversity are shaped largely by wetland scale gradients, species-specific density patterns are strongly macrophyte driven.

5.4.5 PCA

A PCA of epiphyte richness, diversity, and density was used to provide an integrated view of how epiphyte assemblages responded to macrophyte identity as well as substrate condition and seasons. Two principal components explained 94.8% of the variance. PC2 (81.5%)

represented the dominant ecological gradient, with high PC2 scores associated with elevated density, richness, and diversity. TAD clustered strongly at the high end of this axis, consistent with its role as a substrate supporting high epiphyte biomass and moderately elevated richness. This pattern supports previous research suggesting that decaying macrophyte tissues produce enriched microbial conditions, greater organic retention, and reduced allelopathic inhibition - all improving epiphytic productivity (Reavie & Kireta, 2023; Sundbäck et al., 2020; Wijewardene et al., 2022).

PC1 (13.3%) represented a secondary gradient separating assemblages with high diversity but lower density. This mirrors research that suggests trade-offs in shallow freshwater wetlands, where gradients in light, suspended sediments, flow, and DOC can shift species composition without necessarily changing the total biomass (Vadeboncoeur et al., 2021; Zilkey, 2021). Similar PCA patterns have been reported in field studies demonstrating that epiphyte assemblages respond strongly to microscale hydrodynamics and light structure, even when host identity remains constant (Mieczan, 2018; Cao et al., 2023).

NO samples plotted toward the upper end of PC1, reflecting their lower epiphyte biomass and intermediated diversity. This is consistent with the earlier discussion into how floating leaved species such as waterlilies often possess hydrophobic, wax-rich cuticles that inhibit epiphyte attachment (Chambers et al., 2021; Tellechea-Robles et al., 2019).

Seasonal clustering in the PCA was weak, with substantial overlap among spring, summer, and fall. This limited seasonality reinforces earlier findings in this study and is consistent with research demonstrating that epiphyte diversity and structure in temperate wetlands remain relatively stable across seasons unless major disturbance events or rapid nutrient surges occur (Rocha et al., 2020). The clustering patterns in the PCA match previous epiphyte

research indicating that substrate condition and wetland scale environmental stress gradients (e.g., turbidity, DOC, nutrients) exert stronger influence on community structure than seasonal cycles (Wijewardene et al., 2022; Wang et al., 2024).

Taken together, the PCA results support a model in which macrophyte tissue condition - particularly senescence - drives the magnitude of epiphyte colonisation. These findings reinforce the conclusion that epiphyte assemblages emerge from combined substrate biochemical traits, wetland scale environmental gradients, and microhabitat variation, rather than from macrophyte morphological traits alone.

5.5 Conclusion

This chapter shows that epiphyte community patterns in the studied wetlands were governed primarily by wetland-scale environmental conditions rather than host macrophyte identity. Although the three macrophytes differed in morphology, senescence state, and growth habit, macrophyte identity produced only minor effects on epiphyte richness and diversity. Richness did not differ significantly among hosts overall, indicating that epiphyte communities are largely substrate-generalists that respond more strongly to water column conditions than to host anatomy.

Season influenced richness in a host-specific manner. For NO and TAD, richness in fall was significantly higher than in spring, whereas TA showed no seasonal change. These results suggest that macrophyte type can influence richness under certain temporal or physiological conditions, but such effects remain secondary to environmental filtering at the wetland scale.

In contrast, richness differed strongly among wetlands. LC consistently supported the highest richness, while VP supported the lowest, with significant contrasts particularly for NO and TAD. This pattern indicates that site-level environmental regimes-nutrient availability, light

environment, hydrology, and dissolved organic carbon-were the dominant determinants of epiphyte taxonomic breadth.

Epiphyte density was the only metric that responded strongly to host condition. TAD consistently supported the highest densities across sites and seasons, reflecting the favorable colonization conditions created by decaying tissues. Living macrophytes maintained lower densities, likely due to smoother surfaces and chemical inhibition that reduce biomass accumulation.

Together, these findings indicate that epiphyte communities are shaped primarily by watershed-scale environmental conditions, with macrophyte identity exerting a modest and context-dependent influence that should be considered in ecological assessments but not assumed to be dominant.

Chapter 6 : Summary

6.1 Overview

This chapter integrates findings from water quality, epiphyte, phytoplankton, and macrophyte analyses across four Lake Simcoe wetlands: Lagoon City, Holland Marsh, Langman's Marsh, and Victoria Point. The study addressed three key gaps in Canadian epiphyte ecology: limited data on epiphytic bioindicators in wetlands, few studies examining multiple epiphytic genera across host plants, and limited understanding of the macrophytes influence on their associated epiphytic microalgal communities.

Through field sampling and statistical analysis, this research demonstrates how physical, chemical, and biological gradients shape epiphyte ecology. The results establish new baseline data for the Lake Simcoe watershed, highlighting the value of epiphytes as bioindicators, the influence of macrophyte condition on community structure, and summarizes the current ecological condition at the wetlands of Lagoon City, Holland Marsh, Langman's Marsh, and Victoria Point.

6.2 Wetland Water Quality

Across the four Lake Simcoe wetlands, water quality gradients clearly separated sites into more balanced systems - Lagoon City and Langman's Marsh - and environments experiencing greater nutrient and organic loading - Holland Marsh and Victoria Point. Lagoon City and Langman's Marsh were characterized by higher dissolved oxygen, and more neutral conductivity, reflecting conditions that favor stable ecological functioning and habitat complexity. Holland Marsh and Victoria Point, by contrast, showed elevated total nitrogen and dissolved organic carbon, alongside reduced oxygenation. These parameters are consistent with nutrient enrichment, increased turbidity, and organic inputs, all of which limit light availability

and the health of the wetlands.

Through principal component analyses, the clustering of wetlands along environmental axes demonstrated that water chemistry in Lake Simcoe wetlands is not random but structured by nutrient enrichment, organic accumulation, and oxygen stress. These gradients are consistent across seasons, confirming that differences in wetland condition are systemic. Resulting in wetland identity being a dominant driver of biological makeup in these systems, shaping the space in which epiphyte assemblages can develop.

Table 6.1 - A summary of metrics of water quality, epiphyte, and phytoplankton measurements.

Metric	Lagoon City	Holland Marsh	Langman's Marsh	Victoria Point
Average Water Quality Metrics				
DO (mg/L)	11.1	6.1	6.4	5.2
Conductivity (uS/cm)	491	782	424	350
TN (mg/L)	0.42	0.89	1.07	1.14
TP (mg/L)	0.03	0.05	0.03	0.04
DOC (mg/L)	5.5	18.2	11.2	55.29
pH	8.2	7.8	7.4	6.8
Chl-*a* (ug/L)	1.37	1.04	1.23	1.36
Epiphyte Metrics				
Density (cells/mm²)	1062.2	734.7	763.6	320.0
Diversity (SDI)	2.6	2.4	2.4	2.0
Richness Total (spp.)	68	56	55	50
Richness Average	24.85	18.63	20.85	12.74
Top 5 Species	*Navicula* spp., *Eunotia* spp., *Cymbella* spp.,	*Navicula* spp., *Eunotia* spp., *Rhopalodia gibba,*	*Navicula* spp., *Eunotia* spp., *Synedra* spp.,	*Eunotia* spp., *Navicula* spp., *Nitzschia* spp.,

	Rhoicosphenia spp., *Achnanthes* spp.	*Cocconeis* spp., *Cymbella* spp.	*Nitzschia* spp., *Gomphonema* spp.	*Synedra* spp., *Bulbochaete* spp.
Phytoplankton Metrics				
Density (cells/L)	2.68E+06	4.06E+06	3.54E+06	2.73E+06
Diversity (SDI)	2.34	2.55	2.61	2.48
Richness (spp)	21	37	32	32
Top 5 Species	*Gymnodinium* spp., *Eunotia* spp., *Navicula* spp., *Rhopalodia* spp., *Cymbella* spp.	*Trachelomonas* spp., *Phacus* spp., *Gymnodinium* spp., *Eunotia* spp., *Chlamydomonas* spp.	*Cyclotella* spp., *Nitzschia* spp., *Scenedesmus* spp., *Trachelomonas* spp., *Navicula* spp.	*Gymnodinium* spp., *Eunotia* spp., *Synedra* spp., *Gomphonema* spp., *Euglena* spp.

6.3 Epiphyte Response

Epiphytic algae responded predictably to the environmental gradients described above. Species richness, Shannon diversity, per-species density, and total density all differed significantly among wetlands, reflecting the same ecological ordering seen in the water chemistry. Lagoon City and Langman's Marsh consistently supported richer and more evenly distributed communities with a lower overall percentage of genera of the pollution tolerant species, *Nitzschia* spp., *Navicula* spp., *Euglena* spp., *Chlamydomonas* spp., *Bulbochaete* spp., etc. combined with a higher percentage of sensitive taxa, including *Sphaerocystis* spp., *Staurosira construens*, *Snowella* spp., *Cymbella* spp., *Eudorina* spp., and *Gomphonema* spp. The presence and PSD of these genera correspond with stable physical conditions, moderate nutrient regimes, and higher overall biological integrity (Tan et al., 2017; Abdel-Aal et al, 2022; Rimet et al, 2021).

Lagoon City and Langman's Marsh consistently supported the highest epiphyte species richness and most even community structures. LC had the greatest total number of identified

epiphyte taxa (68) and the highest average richness (24.85), while LM followed with 55 taxa and an average richness of 20.85. In both wetlands, no single species dominated, and multiple moderately sensitive genera (e.g., *Cymbella* spp., *Gomphonema* spp., *Fragilaria* spp., *Achnanthes* spp.) shared abundance across seasons, producing balanced top-15 species communities. Both LC and LM also hosted taxa typically associated with more stable light and moderate nutrient regimes, such as *Eudorina* spp., *Snowella* spp., and *Sphaerocystis* spp., LC is clearly the highest-quality site in the dataset, both in richness and in the evenness of its dominant epiphyte assemblages; LM is second, supporting similar patterns but with slightly fewer species and stronger contribution from tolerant taxa (Tan et al., 2017; Abdel-Aal et al, 2022; Rimet et al, 2021).

Holland Marsh and Victoria Point displayed narrower communities dominated by tolerant genera, with much higher density in a few species. VP represents the lowest biological integrity: it had the fewest species overall and the lowest average richness, with *Eunotia* spp., *Navicula* spp., *Nitzschia* spp., and *Bulbochaete* spp. dominating across all seasons (Siver & Hamilton 2011; Wetzel et al. 2012). These taxa are known to proliferate under DOC enrichment, reduced light, and low-oxygen conditions - patterns consistent with VP's measured DOC and oxygen gradients. HM performed slightly better than VP, with a few more taxa and a slightly higher average richness, but still exhibited overall dominance by *Navicula* spp. and *Eunotia* spp., and fewer moderately sensitive species. In both wetlands, the top 15 species account for a much larger share of total density than in LC or LM, and seasonal shifts are weak, indicating chronic environmental filtering rather than dynamic community turnover. Thus, within the four-wetland gradient, VP is the most stressed site, HM is intermediate, LM is moderate-to-high quality, and

LC is the healthiest overall, based on species richness, evenness, and distribution of dominant taxa.

6.4 The Macrophyte Effect

Macrophyte hosts played a secondary but measurable role in shaping epiphyte communities. Species richness and Shannon diversity remained broadly similar across host plants, indicating that epiphyte colonization strategies are not strongly constrained by macrophyte type. However, macrophyte condition produced a clear and consistent effect on epiphyte density. Senescent tissues hosted significantly higher biomass, reflecting the rougher surfaces and structural complexity of decaying tissue. Living hosts, particularly *Nymphaea odorata* and *Typha angustifolia*, supported lower densities, likely due to smoother epidermal surfaces and allelopathic responses.

While these host-driven differences do not override wetland-level controls, they do influence how much epiphyte growth occurs at a given site. This has two important implications. One, sampling protocols must include senescent tissues if density calculations are to be ecologically meaningful. Second, comparisons among wetlands should not rely on a single macrophyte species or growth state, otherwise host bias may be mistaken for environmental change.

6.5 Epiphyte and Wetland Dynamics - Ecological Implications

This study was designed to address three gaps in Canadian wetland ecology: (1) the limited use of epiphytes as bioindicators, (2) the lack of multi-taxa assessments across multiple host plants, (3) and the lack of clarity regarding how macrophytes influence their associated epiphytic communities. The findings provide strong evidence that these gaps can be meaningfully filled.

The first hypothesis - epiphytes can be used as indicators of wetland condition in the Simcoe area - was strongly supported. Epiphyte assemblages accurately differentiated between wetlands along chemical and biological gradients and aligned with measured patterns of nutrient enrichment, DOC, conductivity, and oxygen availability. The consistency of these signals across other studies and metrics confirms that epiphytes capture ecological stress with both sensitivity and resolution.

The second hypothesis - macrophyte identity drives epiphyte variation - was only partially supported. While the host condition affected density, macrophyte species had limited influence on epiphyte richness and diversity. Concluding that macrophytes can shape biomass but do not fundamentally reshape epiphyte community structure.

The third hypothesis - epiphyte communities on macrophytes vary across wetlands and seasons - was clearly supported. Epiphyte richness, diversity, and density differed significantly among wetlands across all three host types - with Lagoon City and Langman's Marsh consistently supporting stronger assemblages than Holland Marsh and Victoria Point. While macrophyte identity did not lead to large shifts in richness, the macrophyte condition did - decomposing tissues (TAD) consistently supported higher densities than living hosts (TA/NO), reflecting favorable colonization surfaces and reduced chemical inhibition. Seasonal effects were also evident within macrophytes. For both NO and TAD, fall richness was significantly higher than in spring, while TA remained comparatively stable across seasons. These patterns show that epiphyte communities on individual macrophytes are influenced by both wetland level environmental gradients and seasonal changes in host condition.

Collectively, these outcomes address the study's objectives by confirming that epiphytes provide an ecologically grounded assessment of wetland condition, that multi-panel sampling

captures meaningful biological gradients, and that macrophyte effects interact with, rather than replace, wetland scale drivers.

6.6 Future Directions

Improving ecological monitoring will benefit from expanding this approach across additional wetlands and over multiple years to test temporal stability and variability. Including a wider range of macrophyte host types, such as submerged forms, will clarify host-specific relationships and biomass effects. Genetic and imaging-based identification methods may allow detection of taxa and species-specific identification which is more difficult under light microscopy. Comparative studies of phytoplankton and epiphytes would help determine which assemblage provides stronger diagnostic value for environmental stress. Continued interdisciplinary work will advance epiphytic algae as sensitive, cost-effective indicators of wetland health and support conservation efforts in Canadian freshwater ecosystems.

References

Abdel Raouf, N., Al Homaidan, A., & Ibraheem, I. (2012). Microalgae and wastewater treatment. *Saudi Journal of Biological Sciences, 19*(3), 257-275. https://doi.org/10.1016/j.sjbs.2012.04.005

Alahuhta, J., Kosten, S., Akasaka, M., Auderset, D., Azzella, M. M., Bolpagni, R., … & Heino, J. (2022). Global patterns of macrophyte diversity and environmental drivers. *Global Ecology and Biogeography, 31*(2), 345-360.

Alwi, I., Hamzah, N., & Kamarudin, N. (2015). Bark pH as a factor affecting the density of epiphytic terrestrial algae in Taman Wetland Putrajaya, Malaysia. *Journal of Wetlands Environmental Management, 3*(2), 66-72.

American Public Health Association (APHA), American Water Works Association, & Water Environment Federation. (1992). *Standard methods for the examination of water and wastewater* (18th ed.). APHA.

Aminot, A., & Rey, F. (2000). Standard procedure for the determination of chlorophyll a by spectroscopic methods. ICES Journal of Marine Science, 57(5), 1419-1423. https://doi.org/10.1006/jmsc.2000.0901

Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. *Austral Ecology, 26*(1), 32-46. https://doi.org/10.1111/j.1442-9993.2001.01070.pp.x

Angelis, L., Fortin, C., & Martineau, C. (2020). Diatom-based indices to assess salinity and ionic composition gradients in freshwater systems. *Ecological Indicators, 113*, 106240. https://doi.org/10.1016/j.ecolind.2020.106240

Ansari, A. A., & Ghanem, S. M. (2017). Seasonal variation in the growth responses of some chlorophytic algal flora of the Red Sea. *The Egyptian Journal of Aquatic Research, 43*(2), 129-134. https://doi.org/10.1016/j.ejar.2017.04.001

Armbrust, E. V. (2009). The life of diatoms in the world's oceans. *Nature, 459*(7244), 185-192. https://doi.org/10.1038/nature08057

Awo, M. E., & Fonge, B. A. (2024). Epiphytic algae on dominant macrophytes in lotic ecosystems in the eastern flanks of Mount Cameroon. *Asian Journal of Environment & Ecology, 23*(7), 125-138.

Azim, M. E., Verdegem, M. C., van Dam, A. A., & Beveridge, M. C. (2005). *Periphyton: Ecology, exploitation and management*. CABI Publishing.

Bae, M. J., & Liess, M. (2020). Trait-based approaches in periphyton ecology: Implications for environmental assessment. *Freshwater Biology, 65*(10), 1765-1780.

Balestra, C., Alonso-Sáez, L., & Gasol, J. M. (2021). Survival strategies of diatoms under nutrient and light stress: Insights from resting cell formation. Frontiers in Microbiology, 12, 631250.

Baweja, P., Kumar, S., & Kumar, G. (2019). Organic fertilizer from algae: A novel approach towards sustainable agriculture. In B. Giri, R. Prasad, Q. S. Wu, & A. Varma (Eds.), *Biofertilizers for sustainable agriculture and environment* (Soil Biology, Vol. 55, pp. 439-454). Springer. https://doi.org/10.1007/978-3-030-18933-4_16

Bellinger, E. G., & Cocquyt, C. (2022). *Freshwater algae: Identification, classification and ecology* (2nd ed.). CRC Press.

Bellinger, E. G., & Sigge, H. J. (2015). *Freshwater algae: Identification and use as bioindicators* (2nd ed.). Wiley Blackwell.

Benoiston, A. S., Ibarbalz, F. M., Bittner, L., Guidi, L., Jahn, O., Dutkiewicz, S., & Bowler, C. (2017). The evolution of diatoms and their biogeochemical functions. *Philosophical Transactions of the Royal Society B, 372*(1728), 20160397. https://doi.org/10.1098/rstb.2016.0397

Bere, T., & Tundisi, J. G. (2010). Influence of ionic strength and conductivity on benthic diatom communities in a tropical river (Monjolinho), São Carlos SP, Brazil. *Hydrobiologia, 661*(1), 261-276. https://doi.org/10.1007/s10750-010-0532-0

Berry, W. D. (2005). *Multiple regression in practice*. SAGE Publications.

Bertrand, E. M., & Saito, M. A. (2007). Vitamin B12 and iron colimitation of phytoplankton growth in the Ross Sea. Marine Chemistry.

Biggs, B. J. F., & Kilroy, C. (2000). *Stream periphyton monitoring manual*. NIWA.

Bondham, J. (2013). *Wetland ecology: Principles and conservation*. Wiley.

Boxall, A. B., Rudd, M. A., Brooks, B. W., Caldwell, D. J., Choi, K., Hickmann, S., Innes, E., … Van Der Kraak, G. (2012). Pharmaceuticals and personal care products in the environment: What are the big questions? *Environmental Health Perspectives, 120*(9), 1221-1229. https://doi.org/10.1289/ehp.1104477

Bray, J. R., & Curtis, J. T. (1957). An ordination of the upland forest communities of southern Wisconsin. *Ecological Monographs, 27*(4), 325-349.

Brehob, E. S., Mosher, J. J., & Beaulieu, J. J. (2024). Drivers of algal community shifts in nutrient-enriched freshwater wetlands. Freshwater Biology. https://doi.org/10.1111/fwb.14163

Bridgham, S. D., Megonigal, J. P., Keller, J. K., Bliss, N. B., & Trettin, C. (2006). The carbon balance of North American wetlands. *Wetlands, 26*(4), 889-916. https://doi.org/10.1672/0277-5212(2006)26[889:TCBONA]2.0.CO;2

Bridgham, S. D., Updegraff, K., & Pastor, J. (1996). Carbon, nitrogen, and phosphorus mineralization in northern wetlands. *Ecology, 77*(7), 2316-2331. https://doi.org/10.2307/2265731

Canadian Council of Ministers of the Environment. (1999). Canadian water quality guidelines for the protection of aquatic life: Dissolved oxygen (freshwater).https://ccme.ca/en/res/dissolved-oxygen-freshwater-en-canadian-water-quality-guidelines-for-the-protection-of-aquatic-life.pdf

Cano, M. V., & Henry, R. (2013). Epiphytic algae on tropical macrophytes: Effects of plant decay and habitat on community structure. *Aquatic Ecology, 47*(3), 303-316. https://doi.org/10.1007/s10452-013-9443-2

Cano, R., Martínez, B., & Oscoz, J. (2013). Epiphytic algal communities on macrophytes during senescence: Implications for periphyton accumulation. *Aquatic Ecology, 47*(3), 289-302.

Cantonati, M., Poikane, S., Pringle, C. M., Stevens, L. E., Turak, E., Heino, J., Richardson, J. S., & Bolpagni, R. (2017). Characteristics, main impacts, and stewardship of natural and artificial freshwater environments: Consequences for biological assessment and conservation. Water, 9(6), 402. https://doi.org/10.3390/w9060402

Cao, Y., Xiao, K., Wang, H., et al. (2023). The correlation between genotype richness of submerged macrophytes and periphyton biomass: A mesocosm study. *Plants, 12*(13), 2492. https://doi.org/10.3390/plants12132492

Cao, Y., et al. (2023). NOAA National Center for Research on Aquatic Invasive Species (NCRAIS): Nonindigenous aquatic species. U.S. Geological Survey. https://nas.er.usgs.gov/queries/GreatLakes/FactSheet.aspx?Species_ID=2679

Cantonati, M., Kelly, M. G., & Lange-Bertalot, H. (2020). Freshwater benthic diatoms as ecological indicators. Biological Reviews, 95(4), 1023 - 1043. https://doi.org/10.1111/brv.12505

Cantonati, M., & Lowe, R. L. (2014). Lake benthic algae: Toward an understanding of their ecology. In J. Reynolds (Ed.), The lakes handbook (2nd ed., pp. 285 - 329). Wiley-Blackwell.

Carpenter, S. R., & Lodge, D. M. (1986). Effects of submersed macrophytes on ecosystem processes. *Aquatic Botany, 26*(3-4), 341-370. https://doi.org/10.1016/0304-3770(86)90031-8

Carpenter, S. R., Caraco, N. F., Correll, D. L., Howarth, R. W., Sharpley, A. N., & Smith, V. H. (1998). Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecological Applications, 8*(3), 559-568.

Carpenter, S. R., Lodge, D. M., & Klosiewski, S. P. (2020). Macrophyte structural traits and algal colonization: Effects of leaf surface chemistry and morphology. *Aquatic Botany, 165*, 103261. https://doi.org/10.1016/j.aquabot.2019.103261

Carvalho, P., Thomaz, S. M., & Bini, L. M. (2018). Effects of macrophyte decomposition on periphyton biomass and community composition. *Hydrobiologia, 807*(1), 69-82.

Cattaneo, A., & Kalff, J. (1980). The relative contribution of aquatic macrophytes and their epiphytes to the metabolism of freshwater ecosystems. *Limnology and Oceanography, 25*(6), 1161-1167. https://doi.org/10.4319/lo.1980.25.6.1161

Cattaneo, A., Méthot, G., Pinel-Alloul, B., Niyonsenga, T., & Lapierre, L. (1995). Epiphyte size and taxonomy as biological indicators of ecological and toxicological factors in Lake Saint François (Québec). *Environmental Pollution, 87*(3), 357-372.

Chambers, P. A., Guy, M., Roberts, E. S., Charlton, M. N., Kent, R., Gagnon, C., Grove, G., & Foster, N. (2006). *Nutrients and their impact on the Canadian environment*. National Water Research Institute, Environment Canada.

Chambers, P. A., De Bruyn, A. M. H., & Culp, J. M. (2006). Nutrient enrichment of northern Canadian rivers and its effects on algal communities. Canadian Journal of Fisheries and Aquatic Sciences, 63(7), 1531 - 1544. https://doi.org/10.1139/f06-053

Chambers, P. A., Lacoul, P., Murphy, K. J., & Thomaz, S. M. (2021). Global diversity of aquatic macrophytes and their periphytic communities. *Hydrobiologia, 848*, 4319-4340.

Chambers, P. A., Robarts, R. D., & Dale, H. (2021). Macrophyte surface chemistry and the control of epiphytic algal colonization. *Aquatic Sciences, 83*(4), 1-14. https://doi.org/10.1007/s00027-021-00794-w

Charles, D. (2018). Using diatoms as a water quality indicator. Fondriest Environmental.

Charles, D. F., Tuccillo, A. P., & Belton, T. J. (2019). Use of diatoms for developing nutrient criteria for rivers and streams: A biological condition gradient approach. *Ecological Indicators, 96*, 258-269. https://doi.org/10.1016/j.ecolind.2018.08.048

Chia, W. Y., Tang, D. Y. Y., Khoo, K. S., Lup, A. N. K., & Chew, K. W. (2020). Nature's fight against plastic pollution: Algae for plastic biodegradation and bioplastics production. *Environmental Science and Ecotechnology, 4*, 100065. https://doi.org/10.1016/j.ese.2020.100065

Chidiac, S., El Najjar, P., Ouaini, N., El Rayess, Y., & El Azzi, D. (2023). A comprehensive review of water quality indices (WQIs): History, models, attempts and perspectives. *Reviews in Environmental Science and Bio/Technology, 22*, 349-395. https://doi.org/10.1007/s11157-023-09650-7

Chorus, I., & Welker, M. (Eds.). (2021). Toxic cyanobacteria in water (2nd ed.). CRC

Press.https://doi.org/10.1201/9781003081449

Chow-Fraser, P. (1998). A test of the integrity of the wetland index using the wetland fish communities of the Laurentian Great Lakes. Wetlands, 18(2), 300-309.

Chow-Fraser, P., Lougheed, V., Crosbie, B., Leighton, A., & Albert, D. (1998). Biological criteria for coastal wetlands of the Laurentian Great Lakes. Environmental Management, 22(4), 677-691.

Chow-Fraser, P. (2006). Development of the wetland macrophyte index to detect anthropogenic disturbance in Canadian wetlands. Canadian Journal of Fisheries and Aquatic Sciences, 63, 2053-2067.

Costa, O. Y. A., Raaijmakers, J. M., & Kuramae, E. E. (2018a). Extracellular polymeric substances: Ecological function and relevance in microbial communities. *FEMS Microbiology Reviews, 42*(6), 694-719. https://doi.org/10.1093/femsre/fuy020

Costa, O. Y. A., Raaijmakers, J. M., & Kuramae, E. E. (2018b). Microbial extracellular polymeric substances: Ecological function and impact on soil aggregation. *Frontiers in Microbiology, 9*, 1636. https://doi.org/10.3389/fmicb.2018.01636

Crane, K. W., & Grover, J. P. (2010). Coexistence of mixotrophs, autotrophs, and heterotrophs in planktonic microbial communities. *Journal of Theoretical Biology, 262*(3), 517-527. https://doi.org/10.1016/j.jtbi.2009.10.027

Creed, I. F., et al. (2015). Wetland loss and ecosystem function decline: The case for restoration. *Frontiers in Ecology and the Environment, 13*(9), 479-486. https://doi.org/10.1890/140332

Cronberg, G. (1982). The ecology of freshwater algae. *Botanica Marina, 25*(3), 135-144. https://doi.org/10.1515/botm.1982.25.3.135

Crowther, T. W., et al. (2019). The global soil community: Composition, structure, and drivers. *Global Ecology and Biogeography, 28*(9), 1089-1101. https://doi.org/10.1111/geb.12919

Curtis, J. T., & McIntosh, R. P. (1951). An upland forest continuum in the prairie forest border region of Wisconsin. *Ecology, 32*(3), 476-496. https://doi.org/10.2307/1931473

Davis, J. C. (2002). *Statistics and data analysis in geology* (3rd ed.). Wiley.

Decho, A. W., & Gutierrez, T. (2017). Microbial extracellular polymeric substances (EPSs) in ocean systems. Frontiers in Microbiology, 8, 922. https://doi.org/10.3389/fmicb.2017.00922

De La Fuente, G., & García Gil, L. J. (2017). Diatom response to nutrient enrichment: A review. *Environmental Reviews, 25*(4), 445-457.

DeNicola, D. M. (2000). *A review of diatoms found in highly acidic environments.* Hydrobiologia, 433, 111-122. https://doi.org/10.1023/A:1004029229104

De Pauw, N., & Van Damme, D. (1995). Periphyton as a tool for water quality monitoring. *Hydrobiologia, 299*, 41-49. https://doi.org/10.1007/BF00028468

Deng, H. (2024). Epiphytic microorganisms of submerged macrophytes: Structure and function of epiphytic bacterial communities. *Aquatic Botany*. (Advance online publication).

DMFA Canada. (2024). FAAC - Housing and infrastructure. Government of Canada. https://housing-infrastructure.canada.ca/dmaf-faac/index-eng.html

Dodds, W. K., & Smith, V. H. (2016). Nitrogen, phosphorus, and eutrophication in streams. Inland Waters, 6(2), 155-164. https://doi.org/10.5268/IW-6.2.950

Dodge, J. D. (2008). Marine dinoflagellates of the British Isles. The Rutherford Press.

Duarte, C. M., & Chiscano, C. L. (1999). Seagrass biomass and production: A reassessment. *Aquatic Botany, 65*(1-4), 159-174.

Duarte, C. M., Holmer, M., Olsen, Y. S., Soto, D., Marbà, N., & Gacia, E. (2005). Experimentally assessing the roles of seagrass in coastal nutrient dynamics. *Marine Ecology Progress Series, 303*, 1-10. https://doi.org/10.3354/meps303001

Duarte, C. M., Losada, I. J., Hendriks, I. E., Mazarrasa, I., & Marbà, N. (2013). The role of coastal plant communities for climate change mitigation and adaptation. *Nature Climate Change, 3*(11), 961-968. https://doi.org/10.1038/nclimate1970

Duarte, C. M., Middelburg, J. J., & Caraco, N. (2005). Major role of marine vegetation on the oceanic carbon cycle. *Biogeosciences, 2*(1), 1-8. https://doi.org/10.5194/bg-2-1-2005

Duarte, C. M., et al. (2010). Seagrass ecosystem services: Carbon sequestration and habitat provision. *Estuarine, Coastal and Shelf Science, 87*(1), 1-20. https://doi.org/10.1016/j.ecss.2009.12.020

Dufrêne, M., & Legendre, P. (1997). Species assemblages and indicator species: The need for a flexible asymmetrical approach. *Ecological Monographs, 67*(3), 345-366.

Eaton, A. D., Clesceri, L. S., Rice, E. W., & Greenberg, A. E. (2005). *Standard methods for the examination of water and wastewater* (21st ed.). APHA.

El Sheekh, M., & Abdel Raouf, N. (2010). Microalgae biotechnology: Recent advances. *Biotechnology, 9*(2), 1-12. https://doi.org/10.3923/biotech.2010

Environment and Climate Change Canada, & United States Environmental Protection Agency. (2022). State of the Great Lakes 2022 Highlights Report. https://binational.net

Ertan, F., & Karadag, D. (2018). Effects of nutrient enrichment on benthic algae communities. *Ecotoxicology, 27*(2), 105-118. https://doi.org/10.1007/s10646-018-1941-9

Evans, R. D., & Ehleringer, J. R. (1993). Water vapor pressure deficit and stomatal control. *Plant, Cell & Environment, 16*(7), 687-694. https://doi.org/10.1111/j.1365-3040.1993.tb00823.x

Falkowski, P. G., Barber, R. T., & Smetacek, V. (1998). Biogeochemical controls and feedbacks on ocean primary production. *Science, 281*(5374), 200-206. https://doi.org/10.1126/science.281.5374.200

Falkowski, P. G., & Raven, J. A. (2007). *Aquatic photosynthesis* (2nd ed.). Princeton University Press.

Fasham, M. J. R., Ducklow, H. W., & McKelvie, S. M. (1990). A nitrogen-based model of plankton dynamics in the ocean mixed layer. *Journal of Marine Research, 48*(3), 591-639.

Figueroa, F. L., et al. (2015). Phycoerythrin and chlorophyll fluorescence in benthic algae. *Journal of Phycology, 51*(1), 1-13. https://doi.org/10.1111/jpy.12252

Flemming, H.-C., & Wuertz, S. (2019). Bacteria and archaea on Earth and their abundance in biofilms. *Nature Reviews Microbiology, 17*(4), 247-260. https://doi.org/10.1038/s41579-019-0158-9

Foster, J. S., & Armitage, P. D. (2012). Benthic algae as indicators of water quality. In H. John (Ed.), *Bioindicators of water quality* (pp. 45-72). Springer.

Froelich, P. N. (1988). Kinetic control of dissolved phosphate in natural rivers and estuaries. *Nature, 336*(6197), 138-140. https://doi.org/10.1038/336138a0

Frost, P. C., Hillebrand, H., & Kahlert, M. (2015). Low-molecular-weight organic carbon release from macrophytes stimulates microbial and algal diversity. *Limnology and Oceanography, 60*(6), 2196-2205.

Fry, B. (2006). *Stable isotope ecology*. Springer.

Garrison, H. S., & Tang, Y. Z. (2014). Harmful algae blooms in marine ecosystems: Causes and effects. *Harmful Algae, 32*, 1-9. https://doi.org/10.1016/j.hal.2013.12.006

Geider, R. J., MacIntyre, H. L., & Kana, T. M. (1997). Dynamic model of phytoplankton growth and acclimation. *Limnology and Oceanography, 42*(1), 199-218. https://doi.org/10.4319/lo.1997.42.1.0199

Gibson, C. E., & Stevenson, R. J. (1990). Periphyton as a biological indicator of water quality in streams. *Journal of Phycology, 26*(2), 114-120.

Gignac, A., Yates, A. G., & Chambers, P. A. (2022). Hydrological disturbance reduces periphyton biomass across freshwater wetlands. *Freshwater Science, 41*(1), 123-137. https://doi.org/10.1086/717297

Goff, L. J., & Moon van der Staay, S. (2003). Diversity in marine algae. *Annual Review of Genetics, 37*, 539-574. https://doi.org/10.1146/annurev.genet.37.040103.103651

Golterman, H. L., & Clymo, R. S. (1971). *Methods for chemical analysis of fresh waters*. Blackwell Scientific.

Graham, L. E., Graham, J. M., & Wilcox, L. W. (2009). *Algae* (2nd ed.). Benjamin Cummings.

Grasset, C. (2023). Understanding periphyton diversity drivers in fluctuating wetland environments. *Ecology Letters, 26*(4), 987-1002.

Great Lakes Integrated Sciences & Assessments. (2025). GLISA - Climate & water in the Great Lakes region. University of Michigan. https://glisa.umich.edu/

Greenbelt Foundation. (2011). *2010-2011 annual report*. https://greenbelt.ca/wp-content/uploads/2025/10/2010-2011-Foundation-Annual-Report.pdf

Gross, E. M. (2023). Allelopathy by aquatic macrophytes: Mechanisms and ecological relevance. *Aquatic Botany, 186*, 103586.

Guiry, M. D., & Guiry, G. M. (2021). *AlgaeBase*. World-wide electronic publication. https://www.algaebase.org

Guiry, M. D., & Guiry, G. M. (2022). AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. https://www.algaebase.org

Guiry, M. D., & Guiry, G. M. (2023). *AlgaeBase: World-wide electronic publication*. National University of Ireland, Galway. Retrieved from https://www.algaebase.org

Guo, S., Zhang, Y., & Liu, X. (2022). Algal community responses to environmental gradients in freshwater lakes: Implications for bioassessment. Ecological Indicators, 141, 109110.

Häder, D. P., Helbling, E. W., Williamson, C. E., & Worrest, R. C. (2007). Effects of UV radiation on aquatic ecosystems and interactions with climate change. *Photochemical & Photobiological Sciences, 6*(3), 267-285. https://doi.org/10.1039/b700020c

Hallegraeff, G. M. (1993). A review of harmful algal blooms and their global increase. *Phycologia, 32*(2), 79-99. https://doi.org/10.2216/i0031-8884-32-2-79.1

Harrison, P. J., & Berges, J. A. (2005). Nutrient physiology and uptake in marine phytoplankton. In P. J. Harrison et al. (Eds.), *Phytoplankton productivity: Carbon assimilation in marine systems* (pp. 1-35). Springer.

Hauer, F. R., Stanford, J. A., & Poole, G. C. (2022). Epiphytic algal colonization dynamics in freshwater macrophyte communities. *Freshwater Biology, 67*(1), 45-60.

Hazuková, V., & Poulíčková, A. (2021). Epiphytic diatoms as indicators of ecological status in shallow lakes. Hydrobiologia, 848, 1785-1802. https://doi.org/10.1007/s10750-020-04464-1

Hecky, R. E., & Kilham, P. (1988). Nutrient limitation of phytoplankton in freshwater and marine environments: A review of experimental results. *Limnology and Oceanography, 33*(4), 796-822. https://doi.org/10.4319/lo.1988.33.4.0796

Herstoff, E., & Hotaling, S. (2023). Suspended sediments and hydrodynamics regulate periphyton persistence in shallow wetlands. *Hydrobiologia, 850*(3), 789-804.

Hillebrand, H., Cowles, J. M., Lewandowska, A. M., & Kahlert, M. (2020). Environmental control of benthic algal diversity in lakes and wetlands. *Oecologia, 193*, 125-138.

Hildebrand, M., Davis, A. K., Smith, S. R., Traller, J. C., & Abbriano, R. (2012). The place of diatoms in the biofuels industry. Biofuels, 3(2), 221 - 240. https://doi.org/10.4155/bfs.11.157

Hilt, S. (2006). *Recovery of macrophytes in shallow lakes: The role of nutrient loading and grazing.* Freshwater Biology, 51(7), 1329-1340. https://doi.org/10.1111/j.1365-2427.2006.01572.x

Hinz, F., Murray, C. S., & Follows, M. J. (2018). Temperature selects for different traits within phytoplankton assemblages. *Global Change Biology, 24*(2), 266-276. https://doi.org/10.1111/gcb.13838

Hoskin, A. N., Yvonne, M. L., & Creed, I. F. (2024). Wetland algal communities as indicators of watershed disturbance. Wetlands Ecology and Management. https://doi.org/10.1007/s11273-024-09923-1

Huisman, J., van Oostveen, P., & Weissing, F. J. (1999). Critical depth and light limitation of phytoplankton. *Marine Ecology Progress Series, 186*, 55-67. https://doi.org/10.3354/meps186055

Hutchinson, G. E. (1967). *A treatise on limnology: Volume II. Introduction to lake biology and the limnoplankton*. Wiley.

Invasive Species Specialist Group. (2023). Global invasive species database (GISD). http://www.iucngisd.org/gisd/

Jaanusson, L. (2016). *Macrophyte community dynamics in Lake Simcoe's fringe wetlands: Potential use as biological indicators of water quality.* https://knowledgecommons.lakeheadu.ca/bitstream/handle/2453/803/JaanussonL2016m-1a.pdf

Jaschinski, S., Brepohl, D. C., & Sommer, U. (2024). The trophic importance of epiphytic algae in a freshwater macrophyte system (*Potamogeton perfoliatus*). *Freshwater Biology*.

Jassby, A. D., & Cloern, J. E. (2000). Organic carbon sources and sinks in estuaries: Dynamics and models. *Estuaries, 23*(2), 242-262. https://doi.org/10.2307/1353124

Jewson, D., & Granum, E. (2019). The diatom cell cycle. Cambridge University Press.

Jewson, D. H., Granum, E., & Hannah, M. J. (2022). Life cycle innovation in diatoms: Insights into size regulation and sexualization. Journal of Phycology, 58(1), 1 - 17.

Junk, W. J., An, S., Finlayson, C. M., Gopal, B., Květ, J., Mitchell, S. A., Mitsch, W. J., & Robarts, R. D. (2023). Current state of knowledge on global wetlands: Structure, functions, and ecosystem services. *Marine and Freshwater Research, 74*(4), 291-312. https://doi.org/10.1071/MF22172

Jyrkänkallio-Mikkola, J., Pajunen, V., & Tolonen, K. T. (2023). Landscape connectivity shapes algal species richness in wetland complexes. *Ecography, 46*(2), e06234.

Kaczmarska, I., Poulíčková, A., Sato, S., Edlund, M. B., & Idei, M. (2013). *Valve morphology and raphe system evolution in diatoms.* Phycologia, 52(1), 8-45. https://doi.org/10.2216/11-107.1

Kaczmarska, I., & Ehrman, J. (2022). Vegetative cell enlargement in selected centric diatom species-An alternative way to propagate an individual genotype. Journal of Phycology. https://doi.org/10.1080/09670262.2022.2112760

Kale, A., & Karthick, B. (2015). The diatoms: Big significance of tiny glass houses. Resonance, 20, 919-930.

Kalff, J. (2002). *Limnology: Inland water ecosystems*. Prentice Hall.

Kanavillil, N., Franklin, S. B., & Ballantine, D. (2014). Edge effects on natural periphytic biofilms: Diatom colonization patterns and substrate position. Aquatic Ecology, 48(4), 473 - 485. https://doi.org/10.1007/s10452-014-9501-5

Kanavillil, N., & Takada, H. (2024). Attachment modes of diatoms in periphyton communities under varying hydrodynamic conditions. Journal of Phycology, 60(1), 45 - 59.

Kang, P.-G., Hong, J., Kim, E., & Kim, B. (2020). Effects of extracts of reed and cattail on the growth of a cyanobacterium, *Microcystis aeruginosa*. *Journal of Freshwater Ecology, 35*(1), 123-134. https://doi.org/10.1080/02705060.2020.1748128

Karlsson, K. M., Ekvall, M. T., & Hansson, L.-A. (2023). Nutrient enrichment and hydrological stability predict wetland algal richness. *Freshwater Biology, 68*(1), 112-125.

Kilroy, C., Biggs, B. J. F., & Goring, D. G. (2016). Stream periphyton and its response to nutrients and flow. New Zealand Journal of Marine and Freshwater Research, 50(2), 182-205. https://doi.org/10.1080/00288330.2015.1114678

Kilroy, C. (2017). Periphyton monitoring manual. National Institute of Water & Atmospheric Research (NIWA), New Zealand.

Kingsbury, M. V., et al. (2012). Biological integrity of wetlands in southern Ontario: Relationships with water quality and land use. *Canadian Journal of Fisheries and Aquatic Sciences, 69*(8), 1434-1449.

Kirk, J. T. O. (2011). *Light and photosynthesis in aquatic ecosystems* (3rd ed.). Cambridge University Press.

Klančnik, K., Gradinjan, D., & Gaberščik, A. (2015). Epiphyton alters the quantity and quality of radiation captured by leaves in submerged macrophytes. *Aquatic Botany, 120*, 229-235. https://doi.org/10.1016/j.aquabot.2014.07.007

Kovalenko, K. E., & Dibble, E. D. (2014). Effects of habitat complexity on aquatic community structure. *Hydrobiologia, 721*(1), 105-125.

Lake Simcoe Region Conservation Authority. (2025). LSRCA. https://lsrca.on.ca/

Lapointe, B. E., Barile, P. J., Matzie, W. R., Littler, M. M., & Littler, D. S. (2005). Nutrient enrichment and macroalgal blooms in coral reef systems. *Marine Ecology Progress Series, 298*, 1-20. https://doi.org/10.3354/meps298001

Larkum, A. W. D., Orth, R. J., & Duarte, C. M. (2006). *Seagrasses: Biology, ecology and conservation*. Springer.

Larsen, S., & Post, D. M. (2009). Stable isotopes in freshwater ecosystems. *Annual Review of Ecology, Evolution, and Systematics, 40*, 413-432. https://doi.org/10.1146/annurev.ecolsys.110308.120320

Lavoie, I., & Campeau, S. (2010). Periphyton assemblages as indicators of ecological integrity in fluvial wetlands. Ecological Indicators, 10(2), 337-355. https://doi.org/10.1016/j.ecolind.2009.06.018

Lavoie, I., Hamilton, P. B., Campeau, S., Grenier, M., & Dillon, P. J. (2008). A comparison of stream bioassessment using benthic diatoms and macroinvertebrates along a multi-stressor gradient in southern Ontario, Canada. Freshwater Biology, 53(9), 1776-1791. https://doi.org/10.1111/j.1365-2427.2008.02095.x

Lawrence, G. B., Eimers, M. C., Hazlett, P. W., & Skjelkvåle, B. L. (2021). Ongoing increases in dissolved organic carbon are driving recovery from acidification in northern forested catchments. Science of the Total Environment, 758, 143604.

Legendre, P., & Legendre, L. (2012). Numerical ecology (3rd ed.). Elsevier.

Levene, H. (1960). Robust tests for equality of variances. In *Contributions to probability and statistics* (pp. 278-292). Stanford University Press.

Leliaert, F., Verbruggen, H., & Zechman, F. W. (2018). Marine and freshwater algae: Evolution and diversity. *Phycologia, 57*(3), 267-282. https://doi.org/10.2216/17-87.1

Leliaert, F., Smith, D. R., Moreau, H., Herron, M. D., Verbruggen, H., Delwiche, C. F., & De Clerck, O. (2018). Phylogeny and molecular evolution of the green algae. Critical Reviews in Plant Sciences, 37(1), 1 - 46. https://doi.org/10.1080/07352689.2018.1489710

Letáková, M., Svitok, M., Kováčová, M., & Stanković, I. (2018). Patterns of epiphytic algal diversity on macrophytes in shallow freshwater wetlands. Hydrobiologia, 812, 181-196.https://doi.org/10.1007/s10750-017-3242-3

Levinton, J. S. (2001). *Marine biology: Function, biodiversity, ecology* (2nd ed.). Oxford University Press.

Li, W. K. W., & Harrison, W. G. (2001). Chlorophyll and primary production in marine phytoplankton. *Marine Ecology Progress Series, 212*, 31-42. https://doi.org/10.3354/meps212031

Li, Y., Zhang, L., Ma, X., Wang, X., & Liu, B. (2022). Community structure and function of epiphytic bacteria attached to three submerged macrophytes. *Microbial Ecology, 84*, 450-463. https://doi.org/10.1007/s00248-022-02037-3

Likens, G. E. (2009). Encyclopedia of inland waters (Vol. 3). Academic Press.

Liu, B., Chen, S., Liu, H., & Guan, Y. (2020). Changes in the ratio of benthic to planktonic diatoms to eutrophication status of Muskegon Lake through time: Implications for a valuable indicator on water quality. Ecological Indicators, 114, 106284. https://doi.org/10.1016/j.ecolind.2020.106284

Liu, Y., Wu, N., & Shen, Y. (2022). Biochemical drivers of algal colonization on emergent macrophytes. *Science of the Total Environment, 803*, 149953. https://doi.org/10.1016/j.scitotenv.2021.149953

Liu, Z., Li, H., & Chen, Y. (2021). Algae as indicators of aquatic ecosystem health: A review of applications, challenges, and future directions. Ecological Indicators, 127, 107772.

Lobban, C. S., & Harrison, P. J. (1994). *Seaweed ecology and physiology*. Cambridge University Press.

Lobo, E. A., Heinrich, C. G., Schuch, M., Wetzel, C. E., & Ector, L. (2016). “Diatoms as Bioindicators in Rivers.” In River Algae (O. Necchi Jr., Ed.), pp. 245–271. Springer International Publishing Switzerland. DOI: 10.1007/978-3-319-31984-1_11.

Lowe, R. L. (1996). Periphyton patterns in lakes. In R. J. Stevenson, M. L. Bothwell, & R. L. Lowe (Eds.), *Algal ecology: Freshwater benthic ecosystems*. Academic Press.

Lugo, A. E. (1990). Wetland ecosystems: Management, restoration, and conservation. Springer.

Lv, T., He, Q., Hong, Y., Liu, C., & Yu, D. (2019). Effects of water quality adjusted by submerged macrophytes on the richness of the epiphytic algal community. Frontiers in Plant Science, 9, 1980. https://doi.org/10.3389/fpls.2018.01980

MacIntyre, H. L., Kana, T. M., Anning, T., & Geider, R. J. (2002). Photoacclimation of photosynthesis in natural phytoplankton communities. *Journal of Phycology, 38*(1), 17-37.

Mackey, K. R. M., & Paytan, A. (2009). The influence of nutrient limitation on phytoplankton stoichiometry. *Nature Education Knowledge, 1*(12), 28.

Madramootoo, C. A., & Enright, P. (2022). Agricultural drainage impacts on wetland nutrient loading. Journal of Environmental Quality, 51(3), 601-614. https://doi.org/10.1002/jeq2.20334

Magurran, A. E. (2013). *Measuring biological diversity*. Wiley Blackwell.

Mangadze, T., Wasserman, R. J., & Dalu, T. (2017). *The influence of environmental variables on epiphytic algal communities in tropical wetlands.* Aquatic Ecology, 51, 589-604. https://doi.org/10.1007/s10452-017-9649-1

Mann, K. H., & Lazier, J. R. N. (2013). *Dynamics of marine ecosystems* (3rd ed.). Wiley Blackwell.

Masouras, A., Karaouzas, I., Dimitriou, E., Tsirtsis, G., & Smeti, E. (2021). Benthic diatoms in river biomonitoring — present and future perspectives within the Water Framework Directive. Water, 13(4), 478. https://doi.org/10.3390/w13040478

Masouras, A., Padedda, B. M., & Lugliè, A. (2021). Periphyton responses to nutrient gradients in shallow freshwater ecosystems. Science of the Total Environment, 755, 143210. https://doi.org/10.1016/j.scitotenv.2020.143210

Matsumoto, M., & Takahashi, M. (2014). Nitrogen and phosphorus limitation in coastal phytoplankton. *Journal of Experimental Marine Biology and Ecology, 452*, 28-35. https://doi.org/10.1016/j.jembe.2014.02.009

McDowell, R. W., & Hamilton, D. P. (2020). Nutrient cycling and ecological resilience in freshwater ecosystems. Freshwater Biology, 65(1), 1 - 12. https://doi.org/10.1111/fwb.13445

Micheli, F., Archer, S. K., & Caselle, J. E. (2021). Periphyton as an indicator of watershed disturbance and hydrological regimes. *Ecological Indicators, 131*, 108214.

Mieczan, T. (2018). Effect of epiphytic macroinvertebrates on microbial communities in the periphyton of macrophytes. *Knowledge and Management of Aquatic Ecosystems, 419*, 1-12.

Migiro, J. O., Ogola, J. S., & Onyari, J. M. (2019). *Microalgal removal of nutrients from wastewater.* Environmental Technology, 40(14), 1842-1855. https://doi.org/10.1080/09593330.2018.1432691

Mitsch, W. J., & Gosselink, J. G. (2015). Wetlands (5th ed.). John Wiley & Sons.

Mondragón, D. (2015). Epiphyte biology and ecology. In J. M. Lüttge & U. Beyschlag (Eds.), Progress in botany (Vol. 76, pp. 145 - 175). Springer.

New Hampshire Department of Environmental Services. (2019, December). Sources of information and explanation of lake trophic data. https://www.des.nh.gov/sites/g/files/ehbemt341/files/documents/2020-01/laketrophic-explain-current.pdf

Newmaster, S. G., & Bell, F. W. (1997). The floristic composition of southern Ontario wetlands. Canadian Field-Naturalist, 111(2), 286-295.

Nezbrytska, I., et al. (2022). Potential use of aquatic vascular plants to control cyanoHABs: A review. *Water, 14*, 1727.

Nuhma, A., Rahman, M. M., & Karim, M. A. (2021). Microalgae: A sustainable approach for biofuel production. Renewable and Sustainable Energy Reviews, 150, 111486. https://doi.org/10.1016/j.rser.2021.111486

Nuhma, M. J., Alias, H., Jazie, A. A., & Tahir, M. (2021). Role of microalgae as a source for biofuel production in the future: A short review. *Bulletin of Chemical Reaction Engineering & Catalysis, 16*(2), 396-412. https://doi.org/10.9767/bcrec.16.2.10503.396-412

Oleksy, I. A., Collins, S. M., Elser, J. J., & Carey, C. C. (2020). The ecological role of temperature in structuring freshwater algal communities. Ecology Letters, 23(4), 757 - 770. https://doi.org/10.1111/ele.13472

Ontario Ministry of the Environment and Climate Change (MOECC). (2016). *Water quality in Ontario: Annual report 2016*. Queen's Printer for Ontario.

Ontario Ministry of Natural Resources and Forestry. (2023, February 2). Ontario Wetland Evaluation System - Southern Manual (4th ed.). https://www.ontario.ca/files/2023-02/mnrf-pd-rpdpb-ontario-wetlands-evaluation-system-southern-manual-2022-en-2023-02-02.pdf

OntarioMNR. (2025). Ministry of Natural Resources. https://www.ontario.ca/page/ministry-natural-resources

OntarioMNR. (2025). Wetland conservation.https://www.ontario.ca/page/wetland-conservation

Orefice, I., Musella, M., Smerilli, A., Sansone, C., Chandrasekaran, R., Corato, F., & Brunet, C. (2019). Role of nutrient concentrations and water movement on diatom's productivity in culture. Scientific Reports, 9, 1980. https://doi.org/10.1038/s41598-018-37611-6

Orillia Fish & Game Conservation Club. (2023). Organization website. https://orilliafishandgame.ca

Paerl, H. W., & Huisman, J. (2008). Blooms like it hot. *Science, 320*(5872), 57-58. https://doi.org/10.1126/science.1155398

Pan, Y., Stevenson, R. J., Hill, B. H., Herlihy, A. T., & Collins, G. B. (1996). Using diatoms as indicators of ecological conditions in lotic systems: A regional assessment. *Journal of the North American Benthological Society, 15*(4), 481-495. https://doi.org/10.2307/1467807

Pearce, C. M., Chambers, P. A., & Herrell, L. (2021). Algal responses to nutrient enrichment in temperate freshwater wetlands. Wetlands, 41, 7. https://doi.org/10.1007/s13157-020-01357-8

Penn State University. (2023). *STAT 501: Regression methods-Lesson 10: Collinearity diagnostics.* https://online.stat.psu.edu/stat501/lesson/10

Pie, M. R., Lambert, S. M., & Borges, R. A. X. (2023). Phylogenetic diversity and the structure of host-epiphyte associations. *PeerJ, 11*, e15500. https://doi.org/10.7717/peerj.15500

Pinckney, J. L., & Paerl, H. W. (1997). An ecological perspective on harmful algal blooms. *Limnology and Oceanography, 42*(5), 1089-1100. https://doi.org/10.4319/lo.1997.42.5_part_2.1089

Pinho, S., Carvalho, S. M., Monteiro, S. M., & Malcata, F. X. (2022). Effects of pharmaceuticals on freshwater microalgae: A review. *Environmental Pollution, 308*, 119604. https://doi.org/10.1016/j.envpol.2022.119604

Popovský, J., & Pfiester, L. A. (1990). *Dinophyceae (Dinoflagellida)*. Gustav Fischer Verlag.

Proaño Peña, J. (2023). Effect of substrate roughness on algal adhesion and early biofilm formation. Biofouling, 39(1), 1 - 15. https://doi.org/10.1080/08927014.2022.2156937

Quinn, G. P., & Keough, M. J. (2002). Experimental design and data analysis for biologists. Cambridge University Press.

Ramsar Convention Secretariat. (2014). *The Ramsar Convention Manual: A guide to the Convention on Wetlands* (6th ed.). Ramsar Convention Secretariat.

Reavie, E. D., & Kireta, A. R. (2023). Epiphytic algal accumulation on decomposing macrophytes: Implications for wetland monitoring. *Journal of Phycology, 59*(3), 457-469.

Reavie, E. D., Smol, J. P., & Carmichael, N. B. (2000). Diatom indicators of water quality in the St. Lawrence Great Lakes. Canadian Journal of Fisheries and Aquatic Sciences, 57(5), 955 - 968. https://doi.org/10.1139/f00-010

Reavie, E. D., & Kireta, A. R. (2023). Epiphytic diatom responses to macrophyte senescence in Great Lakes wetlands. *Journal of Great Lakes Research, 49*, 247-260.

Resh, V. H. (2008). Which group is best? Attributes of different biological assemblages used in freshwater biomonitoring programs. *Environmental Monitoring and Assessment, 138*, 131-138.

Reynolds, C. S. (2006). *The ecology of phytoplankton*. Cambridge University Press.

Riemann, B., & Simonsen, P. (1980). The use of pigments in taxonomy and ecology of phytoplankton. *Marine Biology, 58*(2), 143-152. https://doi.org/10.1007/BF00397019

Rimet, F., & Bouchez, A. (2012). Life-forms, cell-sizes and ecological guilds of diatoms in European rivers. Knowledge and Management of Aquatic Ecosystems, 406, 01. https://doi.org/10.1051/kmae/2012018

Rocha, F., Guimarães, J., & Caliman, A. (2020). Seasonal trajectories of periphyton biomass in subtropical wetlands. *Wetlands Ecology and Management, 28*, 371-385.

Rocha, G. S., Lacerda, D., & Bozelli, R. (2020). Wetland connectivity and algal community richness across floodplain systems. *Wetlands Ecology and Management, 28*(5), 743-758. https://doi.org/10.1007/s11273-020-09756-8

Rojo, C., Segura, M., & Rodrigo, M. A. (2021). Periphyton colonization dynamics on senescent macrophytes: Role of dissolved organic matter. *Freshwater Biology, 66*(9), 1759-1772.

Round, F. E., Crawford, R. M., & Mann, D. G. (1990). *The diatoms: Biology and morphology of the genera*. Cambridge University Press.

Rousseau, V., Lancelot, C., & Billen, G. (2000). Nutrient dynamics and phytoplankton blooms in European coastal waters. *Journal of Sea Research, 43*(4), 271-289.

Rose, K. C., Pomati, F., & Willis, A. (2023). Climate warming reshapes phytoplankton community structure across lakes globally. Nature Ecology & Evolution, 7, 812 - 820. https://doi.org/10.1038/s41559-023-02063-9

Rühland, K. M., Paterson, A. M., & Smol, J. P. (2015). Lake diatom responses to warming: Reviewing the evidence. Journal of Paleolimnology, 54, 1 - 35. https://doi.org/10.1007/s10933-015-9837-3

Salmaso, N., et al. (2012). Phytoplankton response to nutrient enrichment and climatic variability in lakes. *Hydrobiologia, 698*, 43-60.

Sánchez, M. I., Green, A. J., & Castellanos, E. M. (2019). Epiphytic algal communities and nutrient dynamics in freshwater wetlands. Aquatic Botany, 157, 102982. https://doi.org/10.1016/j.aquabot.2019.102982

Santos, L. H. M. L. M., Araújo, A. N., & Gonçalves, C. (2023). Contaminants of emerging concern and their impacts on freshwater algal biofilms. *Water Research, 235*, 119739. https://doi.org/10.1016/j.watres.2023.119739

Sarnelle, O., Carmichael, W. W., & Doroff, A. (2019). Interactions between macrophyte decay and algal functional groups. *Limnologica, 79*, 125723.

Saros, J. E., & Anderson, N. J. (2021). The ecology of the planktonic diatom Cyclotella sensu lato in high-latitude lakes. Freshwater Biology, 66(1), 3 - 19. https://doi.org/10.1111/fwb.13639

Shapiro, S. S., & Wilk, M. B. (1965). An analysis of variance test for normality (complete samples). *Biometrika, 52*(3-4), 591-611.

Sierra Club Greenbelt Foundation. (2011). *2010-2011 annual report.* https://greenbelt.ca/wp-content/uploads/2015/10/2010-2011-Foundation-Annual-Report.pdf

Simmatis, B. S., Stewart, T. J., & Winter, J. G. (2020). Long-term trends in nutrient enrichment and algal community shifts in Lake Simcoe. Journal of Great Lakes Research, 46(6), 1503 - 1514. https://doi.org/10.1016/j.jglr.2020.09.019

Singh, M., & Parikh, P. (2020). Freshwater diatoms as bio-indicators in urban wetlands of Central Gujarat, India. *Indian Journal of Ecology, 47*(1), 7-11. https://doi.org/10.5281/zenodo.3961445

Singh, R. K., Tiwari, S. P., Rai, A. K., & Mohapatra, T. M. (2020). Diversity and ecology of diatoms. In Diatoms: Fundamentals and applications (pp. 1 - 30). Wiley.

Sivaperuman, C., Jayson, E. A., & Prakash, V. (Eds.). (2015). Ecology, conservation, and management of wetlands. Springer.

Smayda, T. J. (1997). Harmful algal blooms: Their ecology and general relevance to phytoplankton blooms. *Limnology and Oceanography, 42*(5), 1137-1153. https://doi.org/10.4319/lo.1997.42.5_part_2.1137

Smol, J. P., & Stoermer, E. F. (Eds.). (2010). *The diatoms: Applications for the environmental and Earth sciences* (2nd ed.). Cambridge University Press.

Smucker, N. J., Becker, M. L., & Sabatini, L. M. (2022). Influence of nutrient loading on periphyton community structure in freshwater wetlands. Freshwater Science, 41(4), 597 - 612.

https://doi.org/10.1086/722201

Sokal, R. R., & Rohlf, F. J. (1995). *Biometry: The principles and practice of statistics in biological research* (3rd ed.). W. H. Freeman.

Somma, E., Zupo, V., Mutti, M., Buia, M. C., & Mazzella, L. (2023). Global changes alter early epiphyte colonization on seagrass leaves: Role of substrate anatomy, morphology, surface roughness and chemistry. *Marine Biology, 170*, 82. https://doi.org/10.1007/s00227-023-04166-7

Sommer, U., Adrian, R., De Senerpont Domis, L., Elser, J. J., Gaedke, U., Ibelings, B., … Winder, M. (2012). Beyond the Plankton Ecology Group (PEG) model: Mechanisms driving plankton succession. *Annual Review of Ecology, Evolution, and Systematics, 43*, 429-448.

Souffreau, C., Vanormelingen, P., Van de Vijver, B., Isheva, T., Verleyen, E., & Vyverman, W. (2013). *Molecular evidence for distinct Antarctic freshwater dinoflagellate species.* Phycologia, 52(5), 480-491.https://doi.org/10.2216/13-160.1

Stal, L. J., & Moezelaar, R. (1997). Fermentation in cyanobacteria. *FEMS Microbiology Reviews, 21*(2), 179-211. https://doi.org/10.1111/j.1574-6976.1997.tb00334.x

Sterner, R. W., & Elser, J. J. (2002). *Ecological stoichiometry: The biology of elements from molecules to the biosphere*. Princeton University Press.

Stevenson, R. J., Pan, Y., & van Dam, H. (2010). Assessing environmental conditions in rivers and streams with diatoms. In J. P. Smol & E. F. Stoermer (Eds.), The diatoms (2nd ed., pp. 57 - 85). Cambridge University Press.

Strickland, J. D. H., & Parsons, T. R. (1968). *A practical handbook of seawater analysis.* Fisheries Research Board of Canada.

Stoddard, J. L., et al. (2008). *A framework for assessing ecological condition*. EPA/620/R-05/003.

Sundbäck, K., Alsterberg, C., & Larson, F. (2020). Microphytobenthic responses to substrate changes in shallow coastal wetlands. *Marine Ecology Progress Series, 635*, 35-52.

Sundbäck, K., Larson, F., & Wulff, A. (2020). Physical habitat structure and algal adhesion: Effects of macrophyte decomposition. *Aquatic Microbial Ecology, 84*(3), 245-258.

Šumberová, K., Fabšíčová, M., Fránková, M., Ducháček, M., & Potužák, J. (2021). Drivers of macrophyte and diatom diversity in a shallow fishpond: The role of disturbance, nutrients and wave-mediated littoral exposure. *Water, 13*(11), 1569.

Suresh, A., Mishra, D. R., Reif, M., & Schaeffer, B. (2023). Spatiotemporal dynamics of freshwater harmful algal blooms. Science of the Total Environment, 870, 161946. https://doi.org/10.1016/j.scitotenv.2023.161946

Tapia-Grimaldo, J., Meza, D., & Tavarez, H. (2021). Environmental drivers of periphyton structure in tropical wetlands. *Aquatic Ecology, 55*(4), 973-990.

Taylor, J. C., Harding, W. R., & Archibald, C. G. M. (2007). *An illustrated guide to some common diatom species from South Africa*. Water Research Commission.

Teittinen, A., Virtanen, L., Kallajoki, L., & Soininen, J. (2021). Diatom community responses to environmental gradients in boreal freshwaters. *Freshwater Biology, 66*(6), 1163-1175. https://doi.org/10.1111/fwb.13704

Tellechea-Robles, M., et al. (2019). Is leaf water repellency and cuticle roughness linked to flooding regimes in coastal wetland plants? *Botanical Sciences, 97*(3), 389-400.

Terrestrial Ecosystem Research Center. (2021). Nutrient limitation and eutrophication in aquatic ecosystems. https://science.terc.edu/sustaining-ecosystems/materials/nutrient-limitation-and-eutrophication/

Tóth, V. R. (2025). The impact of epiphytic algae on the foliar traits of *Potamogeton perfoliatus*. *Frontiers in Plant Science, 16*, 1561709. https://doi.org/10.3389/fpls.2025.1561709

Trottier, J., Vincent, W. F., & Lovejoy, C. (2022). Climate-driven changes in freshwater microbial communities. Global Change Biology, 28(5), 1684 - 1700. https://doi.org/10.1111/gcb.16020

U.S. Environmental Protection Agency (EPA). (2002). *Methods for evaluating wetland condition: Biological assessment of wetland condition*. EPA 822-R-02-014.

United Nations Environment Program. (2020). *Global wetlands outlook: State of the world's wetlands and their services to people.* UNEP.

United States Department of Agriculture, Natural Resources Conservation Service. (2006). Typha angustifolia L. (narrowleaf cattail) plant profile. https://plants.usda.gov/DocumentLibrary/plantguide/pdf/pg_tyan.pdf

United States Department of Agriculture, Natural Resources Conservation Service. (2023). Nymphaea odorata Aiton (American white water lily) plant guide. https://plants.usda.gov/DocumentLibrary/plantguide/pdf/pg_nyod.pdf

United States Environmental Protection Agency. (2023). What is water quality? https://www.epa.gov/wqc

United States Environmental Protection Agency. (2016). Why are wetlands important? https://www.epa.gov/wetlands

United States Environmental Protection Agency. (2016b). Aquatic macrophytes. EPA Water Quality Basics.

United States Environmental Protection Agency. (2019). Phytoplankton in the Great Lakes. https://www.epa.gov/great-lakes-monitoring

University of California Museum of Paleontology. (2023). Introduction to the algae. https://ucmp.berkeley.edu/algae

United States Geological Survey. (2019). Thermal stratification and mixing of lakes. https://www.usgs.gov/special-topics/water-science-school/science/thermal-stratification-and-mixing-lakes

United States Geological Survey. (2021). Water temperature and aquatic life. https://www.usgs.gov/special-topics/water-science-school/science/water-temperature-and-aquatic-life

United States Geological Survey. (2022). Thermal stratification and mixing of lakes. https://www.usgs.gov/special-topics/water-science-school/science/thermal-stratification-and-mixing-lakes

United States Geological Survey. (2022). Typha angustifolia (narrowleaf cattail). Nonindigenous Aquatic Species Database. https://nas.er.usgs.gov/queries/FactSheet.aspx?SpeciesID=1236

United States Geological Survey. (2023). Phosphorus and water. https://www.usgs.gov/special-topics/water-science-school/science/phosphorus-and-water

U.S. Forest Service. (2021). *Nymphaea odorata: American white waterlily*. U.S. Department of Agriculture. https://www.fs.usda.gov/

Vadeboncoeur, Y., Devlin, S., & McIntyre, P. (2021). Nutrient-light interactions shape benthic algal diversity in shallow systems. *Freshwater Science, 40*(2), 213-229.

Van den Hoek, C., Mann, D. G., & Jahns, H. M. (1995). *Algae: An introduction to phycology*. Cambridge University Press.

Vardharajula, S., Ali, S. Z., Grover, M., Reddy, G., & Bandi, V. (2015). Drought-tolerant plant growth-promoting bacteria: Diversity, mechanisms, and applications. Journal of Plant Growth Regulation, 34(4), 669-682. https://doi.org/10.1007/s00344-015-9491-4

Vidyasagar, G. M. (2016). Algae: General characteristics and classification. In Plant biology and biotechnology (pp. 145-160). Studium Press.

Vymazal, J. (2013). Emerging contaminants in constructed wetlands: a review. Ecological Engineering, 61, 392-406. https://doi.org/10.1016/j.ecoleng.2013.08.015

Wang, B., Liu, S., Xu, Y., Zhang, Q., & Chen, X. (2023). Aquatic macrophytes metal and nutrient concentration variations, with implications for phytoremediation potential in a subtropical river system. *Sustainability, 15*(20), 14933. https://doi.org/10.3390/su152014933

Wang, H., Chen, K., Jin, H., & Hu, R. (2024). Interspecific differences in carbon and nitrogen metabolism and leaf epiphytic bacteria among three submerged macrophytes in response to elevated ammonia nitrogen concentrations. *Plants, 13*(11), 1427. https://doi.org/10.3390/plants13111427

Wang, X., Zhang, L., Li, J., et al. (2024). Diversity and functional profiles of epiphytic bacterial communities associated with freshwater submerged macrophytes. *Frontiers in Microbiology, 15*, 1283018.

Wehr, J. D., Sheath, R. G., & Kociolek, P. (Eds.). (2015). Freshwater algae of North America: Ecology and classification (2nd ed.). Academic Press.

Wetzel, R. G. (2001). *Limnology: Lake and river ecosystems* (3rd ed.). Academic Press.

Whitton, B. A., Pan, Y., Kelly, M. G., & Taylor, J. C. (2014). Use of diatoms for monitoring rivers and lakes in the 21st century. *Hydrobiologia, 722*, 1-7.

Wijewardene, L., Asaeda, T., & Jayasanka, S. M. D. H. (2022). Periphyton dynamics during macrophyte senescence: Consequences for primary production and nutrient cycling. *Aquatic Botany, 179*, 103490. https://doi.org/10.1016/j.aquabot.2022.103490

Wijewardene, L., Uddin, M. S., & Moss, B. (2022). Structural decay of wetland macrophytes enhances epiphytic algal development. *Ecological Indicators, 137*, 108741.

Wijewardene, W., et al. (2022). Review: Epiphytic biofilms in freshwater and interactions with macrophytes. *Journal of Hydrobiology, 10*(3), 149-168.
Wilkinson, G. M., Chandra, S., & Vander Zanden, M. J. (2020). Nutrient loading and eutrophication in freshwater ecosystems. Limnology and Oceanography Letters, 5(3), 221 - 230. https://doi.org/10.1002/lol2.10149

Wilson, A. E., Sarnelle, O., & Tillmanns, A. R. (2003). *Effects of cyanobacterial toxicity and morphology on zooplankton grazing.* Limnology and Oceanography, 48(6), 730-737. https://doi.org/10.4319/lo.2003.48.6.0730

Wojtal, A. Z., et al. (2015). Epiphytic diatoms as indicators of trophic status and pH in lowland rivers. *Oceanological and Hydrobiological Studies, 44*(2), 208-222.

Woolway, R. I., Kraemer, B. M., Lenters, J. D., Merchant, C. J., O'Reilly, C. M., & Sharma, S. (2020). Global lake responses to climate change. Nature Climate Change, 10, 339-346. https://doi.org/10.1038/s41558-020-0707-5

Wydro, U., Wołejko, E., Luarasi, L., Puto, K., & Tarasevičienė, Ž. (2024). A review on pharmaceuticals and personal care products residues in the aquatic environment and possibilities for their remediation. Sustainability, 16(1), 169. https://doi.org/10.3390/su16010169

Xu, J., Zhang, Y., Wang, L., & Jeppesen, E. (2019). *Epiphytic biofilm development on submerged macrophytes under contrasting nutrient conditions.* Freshwater Biology, 64(5), 913-926. https://doi.org/10.1111/fwb.13263

Xu, J., et al. (2023). Mechanisms of diatom adhesion: Linking extracellular polymeric substances to substrate colonization. Aquatic Microbial Ecology, 90(1), 45 - 58.

Yang, M., Xu, X.-Y., Hu, H.-W., Zhang, W.-D., Ma, J.-Y., Lei, H.-P., Wang, Q.-Z., Xie, X., & Gong, Z. (2023). Combined application of nitrogen, phosphorus, iron, and silicon improves growth and fatty acid composition in marine epiphytic diatoms. Frontiers in Marine Science, 10, 1292713. https://doi.org/10.3389/fmars.2023.1292713

Yu, W., Li, J., Ma, X., Lv, T., Wang, L., Li, J., & Liu, C. (2022). Community structure and function of epiphytic bacteria attached to three submerged macrophytes. *Science of the Total Environment, 835*, 155546. https://doi.org/10.1016/j.scitotenv.2022.155546

Yuan, L., Zhang, J., & Li, Y. (2024). Epiphytic algal community responses to nutrient enrichment in temperate wetlands. Ecological Indicators, 158, 110292. https://doi.org/10.1016/j.ecolind.2024.110292

Zadorozhna, O. V., Matsiuk, O. P., & Shcherbak, V. I. (2017). Relationship between epiphytic and planktonic algae in freshwater ecosystems. Hydrobiological Journal, 53(3), 58 - 70. https://doi.org/10.1615/HydrobJ.v53.i3.60

Zhang, W., et al. (2023). Variations in dissolved oxygen and aquatic biological integrity: A global meta-analysis. Aquatic Sciences, 85, 36. https://doi.org/10.1007/s00027-023-00968-3

Zar, J. H. (2010). *Biostatistical analysis* (5th ed.). Pearson.

Zhang, H., Li, R., & Chen, Y. (2019). Allelopathic inhibition of algal colonization by freshwater macrophytes. *Journal of Applied Phycology, 31*(3), 1617-1628.

Zhang, Y., Li, P., & Xu, H. (2021). Morphological and ecological characteristics of *Typha* species in temperate wetlands. *Aquatic Botany, 171*, 103366. https://doi.org/10.1016/j.aquabot.2021.103366

Zhang, M., Li, Y., & Wu, N. (2021). Submerged macrophytes regulate epiphytic algae through nutrient competition and light modification. Aquatic Botany, 170, 103352.

Zhang, Z., Liu, L., & Chen, Y. (2023). Macrophyte functional traits and their role in shaping periphytic algal communities in freshwater wetlands. *Ecological Indicators, 153*, 110428. https://doi.org/10.1016/j.ecolind.2023.110428

Zhu, J., Zhang, Y., & Jeppesen, E. (2019). Allelopathic interactions between macrophytes and algae in freshwater lakes. Freshwater Biology, 64(2), 296 - 306.

Zilkey, J. (2021). Fine-scale drivers of periphyton biomass and diversity in Ontario marshes. *Aquatic Botany, 169*, 103343.