MACROLICHEN COMMUNITY STRUCTURE IN BOREAL FORESTED WETLANDS ON THE ISLAND OF NEWFOUNDLAND, CANADA

By

© Tegan Alienor Florence Padgett

A thesis submitted to the School of Graduate Studies in partial fulfilment

of the requirements for the degree of

Master of Science
Department of Biology
Memorial University of Newfoundland
May 2019
St. John’s, Newfoundland and Labrador

ABSTRACT

Forested wetlands provide important ecosystem services and vital habitat for numerous organisms. Epiphytic macrolichens are a common and abundant group of organisms in forested wetlands and, given their habitat specificity, they are of potential use as indicators of forested wetlands and spatial boundaries. However, little is known about the community structure of macrolichens in forested wetlands. To address this, I first tested for differences in macrolichen communities and habitat associations between wetlands and ecoregions. I found significant differences between forested wetland classes and ecoregions and identified potential indicator species. Second, I tested for differences in macrolichen communities among swamps, ecotones, and adjacent upland forests. I found that macrolichen community richness and diversity were highest in swamps and lowest in upland forests, and that macrolichen communities were significantly different among swamps, upland forests, and their ecotones. The results of this research highlight the potential use of macrolichens as indicators of forested wetlands and their spatial boundaries.

ACKNOWLEDGEMENTS

I thank my supervisor Dr. Yolanda Wiersma for providing support, guidance, and giving me independence to figure out my way. I also thank my committee members, Dr. Karen Harper and Dr. Carissa Brown, for their feedback and expertise. Also, thanks go to my field assistants John Godfrey and Brian Ronayne for all their hard work, and the Landscape Ecology and Spatial Analysis lab members for general support and feedback. A big thank you is due to Travis Heckford for editing, maps, and support throughout this time. I thank the Nature Conservancy of Canada for allowing me access to their land for research purposes, and the Province of Newfoundland and Labrador for issuing permits and allowing me to use Salmonier Nature Park for accommodations and land for research. Jean Granger and Dr. Bahram Salehi provided wetland information and Mac Pitcher assisted with lichen identification. Mac Pitcher and Laura Park also provided accommodations. Finally, I thank the funding received through Graduate Studies Fellowships and Teaching Assistantships at Memorial University of Newfoundland, Department of Biology, as well a grant from the American Bryological and Lichenological Society. This work was funded by Environment and Climate Change Canada Atlantic Ecosystems Initiatives and supported by Dr. Wiersma's NSERC grant.

Table of Contents

ABSTRACT i

ACKNOWLEDGEMENTS ii

List of Tables v

List of Figures vii

List of Appendices ix

Chapter 1 Introduction and Overview 1

- 1.1 Introduction 1
- 1.2 Study Areas 4
- 1.3 Chapter Objectives and Hypotheses 6
- 1.4 References 7
- 1.5 Co-authorship Statement 10

Chapter 2 Macrolichen community composition and habitat associations in boreal forested wetlands 11

- 2.1 Abstract 11
- 2.2 Introduction 12
- 2.3 Methods 14
  - 2.3.1 Study Areas 14
  - 2.3.2 Site Selection 17
  - 2.3.3 Study Design 17
  - 2.3.4 Data Analysis 19
- 2.4 Results 21
- 2.5 Discussion 29
- 2.6 References 33

Chapter 3 Macrolichen richness, diversity, and composition in boreal forested swamps, ecotones, and upland forests 38

- 3.1 Abstract 38
- 3.2 Introduction 39
- 3.3 Methods 41

        - 3.3.1 Study Areas..41
        - 3.3.2 Site Selection 43
        - 3.3.3 Study Design..43
        - 3.3.4 Data Analysis...45
    - 3.4 Results 47
    - 3.5 Discussion 55
    - 3.6 References 57
- Chapter 4 Summary 60
    - 4.1 Summary of Results 60
    - 4.2 Limitations 62
    - 4.3 Conservation Implications and Future Research...64
    - 4.4 References 66
- Appendices..68

List of Tables

Table 2-1. Characteristics of the three forested wetland classes (bog, fen, swamp) including soil pH, canopy closure, the dominant tree species in the plot, and the dominant vegetation in the shrub and ground layer. ANOVAs were used to compare means with the significance set at α = 0.05...22

Table 2-2. Macrolichen thalli count by forested wetland class and ecoregion with relative abundance (%) in brackets. 23

Table 2-3. Explanatory variable vectors fitted to the non-metric multidimensional (NMDS) scaling analysis plot. The number of permutations was 999 and the significance level was α = 0.05. The NMDS 1 and 2 columns give the cosines of the vectors, and the R^2 values give the squared correlation coefficient. DBH = diameter at breast height.......27

Table 2-4. Results from the non-parametric multivariate analysis of variance (perMANOVA) using Bray-Curtis distance with 999 permutations and a square-root transformed distance matrix. Data were modified to have a balanced design by averaging the Central Newfoundland sites together and significance was α = 0.05. 27

Table 2-5. Significant associations of macrolichens with forested wetlands and ecoregions (maximum habitat association); the observed Indicator Values show the strength of the association, along with a *p*-value (α = 0.05). 28

Table 3-1. Characteristics of the three transect types (swamp, ecotone, upland) showing mean canopy closure, tree age, tree diameter at breast height (DBH), and tree height. ANOVAs were used to compare means with the significance set at α = 0.05. Each transect has an n = 15 except for tree age (n = 30) as two trees were cored per transect. Values shown are mean (±SE). 48

Table 3-2. Macrolichen thalli count with relative abundance (%) in brackets and the transect type lichen species are predominately association with. Significant lichen associations ($\alpha = 0.05$) are indicated with an asterisk..49

Table 3-3. Explanatory variable vectors fitted to the non-metric multidimensional (NMDS) scaling analysis plot. The number of permutations was 999 and the significance level was $\alpha = 0.05$. The NMDS 1 and 2 columns give the cosines of the vectors, and the R^2 values give the squared correlation coefficient. DBH = diameter at breast height.......54

Table 3-4. Results of the non-parametric multivariate analysis of variance (perMANOVA) using Bray-Curtis dissimilarity for macrolichen community composition by transect type (swamp, ecotone, upland). Significance level was $\alpha = 0.05$). 54

List of Figures

Figure 2-1. Map of the forested wetland (bog, fen, swamp) sites showing the ecoregions of Newfoundland. There were nine forested wetland sites in the Northern Peninsula Ecoregion, eighteen sites in the Central Newfoundland Ecoregion, and nine sites in the Avalon Forest Ecoregion. 16

Figure 2-2. Boxplots comparing Shannon Diversity Index for lichens by a) ecoregion and b) forested wetland class. There were nine study sites in the Avalon Forest, 18 sites in Central Newfoundland, and nine in the Northern Peninsula. Kruskal-Wallis tests compared the differences in richness and diversity among ecoregions and forested wetland classes. Boxes represent 25th and 75th quartiles, and whiskers represent the minimum and maximum data points, exclusive of outliers. 24

Figure 2-3. Ordination results of the non-metric multidimensional scaling (NMDS) analysis with a) ecoregions and b) forested wetland classes. A final stress of 0.117 for a three-dimensional solution was concluded. Each data point represents a site and ellipses represent 95% confidence intervals around each centroid. Environmental site variables were fitted as vectors with the vector arrows position indicating the direction of the gradient and length indicating the strength of the gradient...26

Figure 3-1. Map of study areas showing the 15 sites within the Avalon Forest Ecoregion. One site is just outside of the ecoregion boundary because of being on the private land of a partner on the project...42

Figure 3-2. Macrolichen richness boxplots displayed by transect type. A Kruskal-Wallis test compared the difference among the three transect types, and pairwise comparisons were used to detect differences between each transect type. Significance levels were set to α = 0.05. Boxes represent 25th and 75th quartiles, and whiskers represent the minimum and maximum data points, exclusive of outliers. 51

Figure 3-3. Macrolichen Shannon diversity index boxplots displayed by transect type. A Kruskal-Wallis test compared the difference among the three transect types. Significance levels were set to α = 0.05. Boxes represent 25th and 75th quartiles, and whiskers represent the minimum and maximum data points, exclusive of outliers. 52

Figure 3-4. Ordination results of the non-metric multidimensional scaling (NMDS) analysis of the macrolichen community composition with 95% confidence interval ellipses around each transect type. A final stress of 0.123 with a three-dimensional solution was concluded. Environmental site variables were fitted as vectors with the vector arrows position indicating the direction of the gradient and length indicating the strength of the gradient. 53

List of Appendices

Appendix 1. Supplemental map showing the spatial distribution of chapter 2 sample sites. 68

Appendix 2. Supplement information on geographic locations of chapter 2 sample sites. Abbreviations for study sites are as follows: AVL = Avalon, GFW = Grand Falls-Windsor, DLK = Deer Lake, GMN = Gros Morne. Abbreviations for ecoregions are as follows: AF = Avalon Forest, CN = Central Newfoundland, NP = Northern Peninsula...69

Appendix 3. Representative site photos of forested bogs, fens, and swamps and their ground and shrub cover from chapter 2. 71

Appendix 4. Supplement information on geographic locations of chapter 3 sample sites. 74

Appendix 5. Representative site photos of the swamp, ecotone, and upland forest transect areas from chapter 3. 76

Chapter 1 Introduction and Overview

1.1 Introduction

Forested wetlands are one of the most understudied ecosystems in the world despite being common (Sjöberg and Ericson, 1997). Wetlands cover up to 6% of the Earth's surface and provide important ecosystem services such as nutrient cycling, water purification, flood mitigation, and habitat for a multitude of organisms (Smith et al., 2007). The definition of forested wetlands differs per country but are generally defined as heterogeneous forest types characterized by a high-water table of variable duration, which can vary in hydrology, canopy closure, and vegetation cover (National Wetlands Working Group, 1997). Forested wetlands are susceptible to threats such as timber harvest, land development, wind-throw, and disease outbreaks, and because of these threats, need proper management (Keeping and Hanel, 2006).

Of the five main wetland classes in Canada, three can be forested: bog, fen, and swamp. On the island of Newfoundland, bogs receive water exclusively from precipitation creating an acidic sphagnum moss and ericaceous plant-dominated ecosystem; black spruce (*Picea mariana*) and tamarack (*Larix laricina*) can be present in either shrub or tree form. Fens are fed by groundwater and have a high dissolved mineral content, dominant graminoid (grass-like plants) vegetation and mainly black spruce with some tamaracks present. Swamps typically have strong but seasonally variable surface water influences and high canopy cover. High canopy cover and associated shade leads to a predominance of mosses and ferns on the forest floor. Swamps have a higher diversity of tree species, usually consisting of balsam fir (*Abies balsamea*), black spruce, and tamarack (National Wetlands Working Group, 1997).

Wetland management is increasingly important as land conversion increases the effects of climate change (Zedler and Kercher, 2005). Classifying and delineating wetlands are fundamental components of management and can be challenging. Many of the challenges are due to wetland habitats not fitting into the designed classes or vague boundaries with adjacent habitats. Forested wetlands have the added challenge of their forest canopy obscuring wetland attributes, such as hydrology, making imagery classification difficult (Sader et al., 1995). On-the-ground classification is important for forested wetlands, even with the availability of advanced technologies that can facilitate automated classification, such as Light Detection and Ranging, imagery, and models (Maxa and Bolstad, 2009; O'Neil et al., 2018). Along with hydrophytic vegetation and other wetland indicators, it appears that epiphytic lichens have potential as indicators of the presence of forested wetland conditions.

Indicator species can be used to identify different habitats or areas. They are defined as organisms whose presence reflects the conditions of the environment where they are found (Siddig et al., 2016). Because of this, indicator species can be used as wetland indicators if they reflect natural wetland processes and provide evidence of functioning wetlands (Tiner, 2017). Wetland indicator species can be diverse and are found in many different microhabitats of the wetland such as in the soil or water, on the ground, or as epiphytes living on other plants such as trees. Compared to non-forested wetlands, forested wetlands have an additional vertical growing surface for epiphytes in the form of trees. Lichens, for example, can take advantage of this substrate and use the trees to grow on. The relationship between epiphytic lichen and the trees of forested wetlands could potentially lead to highlighting some forested wetland lichen indicator species.

Lichens are an important ecological component of forested wetland habitat and contribute to its biodiversity, biomass, and nutrient levels (Kuusinen, 1996). Lichens are composite organisms made up of fungal components, sometimes from one or two fungi (Spribille et al., 2016), and photosynthesizing components such as algae and or cyanobacteria. There are many forms of lichens. They have been classified into different groups depending on growth form, with macrolichens encompassing the fruticose and foliose growth forms (Brodo et al., 2001). Lichens play important ecological roles, many of which are not well understood. They provide food, shelter to microorganisms, and nutrients to ecosystems and can be used as bio-monitors for pollution levels. Lichens do not have a vascular system, instead they absorb atmospheric moisture and particles through their cortex (Purvis, 2000). This characteristic allows them to be used to monitor air pollution and ecosystem integrity (McMullin et al., 2017; Nimis and Purvis, 2002; Seed et al., 2013).

Biodiversity plays a vital role in the integrity of ecosystems with higher biodiversity creating more resilient systems (Peterson et al., 1998). Wetlands often have higher biodiversity than non-wetland habitats (Flinn et al., 2008). Lichens contribute greatly to forest biodiversity and ecosystem dynamics. They contribute high species richness, sometimes even higher than vascular plants (Affeld et al., 2008), and can have high levels of biomass, which contributes to forest nutrient cycles (Ellis, 2012). Species diversity can also be used to highlight important habitats and, because lichens can reflect changes in their environment, they can be used as diversity indicators for important areas of conservation (Kuusinen, 1996). I would expect lichen diversity to differ more between forested wetland classes within a region than across regions. The difference in canopy cover and wetland hydrology between forested wetland classes could have a strong influence on the lichen communities living on the trees of forested wetlands. Also, I expect to see lichen diversity to be higher in forested wetlands compared to upland non-wetland forests, following a similar pattern to vascular plants (Flinn et al., 2008). This is because lichens are poikilohydric and rely on atmospheric moisture for activities such as photosynthesis. The more humid forested swamp could create a more desirable habitat than the drier upland forest (Gauslaa, 2014).

1.2 Study Areas

I conducted this study on the island of Newfoundland, Canada. Newfoundland is part of the boreal forest but uniquely shaped by oceanic and continental influences. This leads to Newfoundland having an uncharacteristic disturbance history compared to other parts of the Canadian boreal forest (Arsenault et al., 2016). There is considerable variation in geology, climate, landscape character and vegetation structure across Newfoundland. There are nine ecologically distinct areas, called ecoregions (Damman, 1983), and I used three for this study: Avalon Forest, Central Newfoundland, and Northern Peninsula Forest.

The Avalon Forest Ecoregion is located in the middle of the southeastern peninsula of Newfoundland and is the smallest ecoregion, covering about 500 km^2. The topography in the Avalon Forest is irregular, with ribbed moraines and numerous small water bodies resulting in a high diversity of habitat conditions within the ecoregion. With more habitat diversity, the Avalon Forest houses a greater variety of vegetation including balsam fir-dominated forests, with birch (*Betula* spp.) and spruce (*Picea* spp.). There could be a higher number of swamps due to a large amount of tree cover and lowland topography. The climate is highly influenced by its proximity to the ocean and its sheltered position on the island with fog frequently occurring, especially in the summers (Damman, 1983). This humid environment creates an ideal habitat for lichens and has been shown to have high lichen species richness compared to other parts of the island (McMullin and Wiersma, 2017). I expect to see some lichen species that have affinities for coastal and more humid areas to be most common within the Avalon Forest Ecoregion.

The Central Newfoundland Ecoregion is located in the north central part of the island. This ecoregion is heavily forested and is a typical boreal forest with a mix of spruce, fir, and deciduous trees species (Meades and Moore, 1989). The climate is the most continental of any part of the island meaning it usually has higher summer and lower winter temperatures. Due to the higher summer temperatures and high evaporation rates, the Central Newfoundland Ecoregion is one of the driest parts of the island. This dryness allows black spruce-lichen forests to occur more frequently than other parts of the island (Damman, 1983). I expected the Central Newfoundland Ecoregion to have lichen communities that were less similar than those in the other two ecoregions because of its unique inland setting and associated drier climate.

The Northern Peninsula Forest Ecoregion is the northwestern part of the island. It is dominated by balsam fir, with black spruce more common at the higher elevations. The topography is diverse, creating many different habitats and a wider range of vegetation niches (Bouchard et al., 1991). The climate is marine-influenced with cooler summers creating a shorter growing season than most parts of the island. Lower summer temperatures result in less evaporation, higher soil moisture and more humid forests (Damman, 1983). This ecoregion could potentially show similar patterns in macrolichen community composition with the Avalon Forest Ecoregion due to both regions being coastal.

1.3 Chapter Objectives and Hypotheses

The objective of the second chapter was to determine what macrolichens are found in different forested wetland classes and whether and how they differ regarding diversity and composition. I investigated variation in macrolichen community composition between different forested wetland classes and across ecoregions and identified potential macrolichen indicator species for classes and ecoregions. I hypothesized that 1) macrolichen community composition would vary between forested wetland class more than across ecoregions, and 2) macrolichen indicator species exist for forested wetland classes and ecoregions. The results of this chapter address knowledge gaps of forested wetland macrolichen spatial patterns and habitat associations.

The objective of the third chapter was to determine whether macrolichen richness, diversity, and community composition differed between forested swamps and non-wetland upland forests. I compared macrolichen community composition in forested swamps, ecotones, and adjacent upland forests to test the following predictions of 1) macrolichen richness and diversity is highest in forested swamps, but alternatively 1a) ecotones have the highest richness and diversity, and 2) macrolichen community composition of forested swamps and upland forests is distinct and overlaps with ecotones. The results of this chapter will indicate differences in forested habitats and highlight areas of high lichen biodiversity. Overall, this thesis will provide baseline data of what macrolichens are found in different forested wetland classes, associated indicators, and diversity levels across regions, while at the same time testing some questions about niche theory and spatial distribution patterns.

1.4 References

Affeld, K., Sullivan, J., Worner, S.P., and Didham, R.K. (2008). Can spatial variation in epiphyte diversity and community structure be predicted from sampling vascular epiphytes alone? Journal of Biogeography 35: 2274–2288.

Arsenault, A., Leblanc, R., Earle, E., Brooks, D., Clarke, B., Lavigne, D., and Royer, L. (2016). Unravelling the past to manage Newfoundland's forests for the future. The Forestry Chronical 92: 487-502.

Bouchard, A., Hay, S., Bergeron, Y., and Leduc, A. (1991). The vascular flora of Gros Morne National Park, Newfoundland: A habitat classification approach based on floristic, biogeographical and life-form data. In Quantitative Approaches to Phytogeography (pp. 183–204). Dordrecht: Springer.

Brodo, I.M., Sharnoff, S.D., and Sharnoff, S. (2001). Lichens of North America. Yale University Press, New Haven.

Damman, A. W. H. (1983). An ecological subdivision of the island of Newfoundland. In G. R. South (Ed.), Biogeography and Ecology of the Island of Newfoundland (pp. 163–206). The Hague, the Netherlands: Dr. W Junk Publishers.

Ellis, C.J. (2012). Lichen epiphyte diversity: a species, community and trait-based review. Perspectives in Plant Ecology, Evolution and Systematics 14: 131–152.

Flinn, K.M., Lechowicz, M.J., and Waterway, M.J. (2008). Plant species diversity and composition of wetlands within an upland forest. American Journal of Botany 95: 1216–1224.

Gauslaa, Y. (2014). Rain, dew, and humid air as drivers of morphology, function and spatial distribution in epiphytic lichens. The Lichenologist 46: 1–16.

Keeping, B. and Hanel, C. (2006). A 5 Year (2006 – 2011) Management plan for the Boreal Felt Lichen (*Erioderma pedicellatum*) in Newfoundland and Labrador.

Kuusinen, M. (1996). Importance of spruce swamp-forests for epiphyte diversity and flora on *Picea abies* in southern and middle boreal Finland. Ecography 19: 41–51.

Maxa, M. and Bolstad, P. (2009). Mapping northern wetlands with high resolution satellite images and LiDAR. Wetlands 29: 248–260.

McMullin, R.T., Ure, D., Smith, M., Clapp, H., and Wiersma, Y.F. (2017). Ten years of monitoring air quality and ecological integrity using field-identifiable lichens at Kejimkujik National Park and National Historic Site in Nova Scotia, Canada. Ecological Indicators 81: 214–221.

McMullin, R.T. and Wiersma, Y.F. (2017). Lichens and allied fungi of Salmonier Nature Park, Newfoundland. The Journal of the Torrey Botanical Society 144: 357–369.

Meades, W.J. and Moore, L. (1989). Forest Site Classification Manual. A guide to the Damman forest types of Newfoundland. Forest Resource Development Agreement Report 003.

National Wetlands Working Group (1997). The Canadian wetland classification system. Waterloo, Canada: Wetlands Research Branch, University of Waterloo.

Nimis, P.L. and Purvis, O.W. (2002). Monitoring lichens as indicators of pollution. In P. L. Nimis, C. Scheidegger, P. A. Wolseley (Eds.), Monitoring with Lichens - Monitoring Lichens (pp. 7–10). Dordrecht: Springer.

O'Neil, G.L., Goodall, J.L., and Watson, L.T. (2018). Evaluating the potential for site-specific modification of LiDAR DEM derivatives to improve environmental planning-scale wetland identification using Random Forest classification. Journal of Hydrology 559: 192–208.

Peterson, G.D., Allen, C.R., and Holling, C.S. (1998). Ecological Resilience, Biodiversity, and Scale. Ecosystems 1: 6–18.

Purvis, W. (2000). Lichens. Smithsonian Institution Press in association with the Natural History Museum, London., The Natural History Museum, London.

Sader, S.A., Ahl, D., and Liou, W.S. (1995). Accuracy of landsat-TM and GIS rule-based methods for forest wetland classification in Maine. Remote Sensing Environment 53: 133–144.

Seed, L., Wolseley, P., Gosling, L., Davies, L., and Power, S.A. (2013). Modelling relationships between lichen bioindicators, air quality and climate on a national scale: results from the UK OPAL air survey. Environmental Pollution 182: 437–447.

Siddig, A.A.H., Ellison, A.M., Ochs, A., Villar-Leeman, C., and Lau, M.K. (2016). How do ecologists select and use indicator species to monitor ecological change? Insights from 14 years of publication in Ecological Indicators. Ecological Indicators 60: 223–230.

Sjöberg, K. and Ericson, L. (1997). Mosaic boreal landscapes with open and forested wetlands. Ecological Bulletins 46: 48–60.

Smith, C., Morissette, J., Forest, S., Falk, D., and Butterworth, E. (2007). Synthesis of technical information on forest wetlands in Canada. Technical Bulletin 938: 1–97.

Spribille, T., Tuovinen, V., Resl, P., Vanderpool, D., Wolinski, H., Aime, M.C., Schneider, K., Stabentheiner, E., Toome-Heller, M., Thor, G., Mayrhofer, H., Johannesson, H., and McCutcheon, J.P. (2016). Basidiomycete yeasts in the cortex of ascomycete macrolichens. Science 353: 488–491.

Tiner, R. (2017). Wetland Indicators: A guide to wetland formation, identification, delineation, classification, and mapping (Second Edi). Boca Raton: Taylor & Francis.

Zedler, J.B. and Kercher, S. (2005). Wetland Resources: status, trends, ecosystem services, and restorability. Annual Review of Environment and Resources 30: 39–74.

1.5 Co-authorship Statement

This research was supervised by Dr. Yolanda Wiersma of Memorial University of Newfoundland, Department of Biology. As the primary author, I was responsible for all data collection and analysis for both data chapters (chapter 2 and 3), and most of the thesis writing. Chapter 2, titled "Macrolichen community composition and habitat associations in boreal forested wetlands", is co-authored by Dr. Wiersma who helped refine the hypotheses and study design, and assisted in the revisions. Chapter 3, titled "Macrolichen richness, diversity, and composition in boreal forested swamps, ecotones, and upland forests", is co-authored by Dr. Wiersma who helped refine the hypotheses and assisted in the revisions. Chapter 2 is currently in preparation for submission to the peer-reviewed journal *The Bryologist* and chapter 3 will be submitted to the peer-reviewed journal *Biodiversity and Conservation* in the coming weeks.

Chapter 2 Macrolichen community composition and habitat associations in boreal forested wetlands

2.1 Abstract

Epiphytic lichen communities are an abundant component of boreal forested wetland ecosystems. However, little is known about these lichens' ecology or how lichen communities interact with different forested wetlands. Lichens, due to their sensitivity to smaller-scale influences such as the immediate surroundings of their habitats, have been used as indicator species in forested systems and could be potentially used as indicator species for forested wetland classification. I tested the potential for macrolichens to act as indicators of forested bog, fen, and swamp wetland classes in three ecoregions on the island of Newfoundland, Canada. Macrolichen thalli were counted, by species, on the lower bole of black spruce (*Picea mariana*) trees within plots from each forested wetland class in each ecoregion. I also collected data on habitat characteristics in each wetland including soil pH, canopy closure, ground and shrub cover, which differed significantly among forested wetland classes. Macrolichen communities differed among ecoregions and forested wetland classes but differed more between ecoregions. Furthermore, I identified potential lichen indicator species for forested wetland classes and ecoregions.

2.2 Introduction

Wetlands play important ecological and economic roles. In addition to providing habitat for many organisms, they provide important ecosystem services such as flood mitigation, water filtration, and pollution control (Barbier et al., 1997). However, many threats, such as land development and, more specifically, drainage for agriculture, are degrading wetlands globally and the need for wetland management is increasingly important (Zedler and Kercher, 2005). Species surveys, classification, and delineation are key components of wetland management (Finlayson and van der Valk, 1995; U.S. Army Corps of Engineers, 2011) and are important for wetland inventories, designating buffer zones, and to produce accurate wetland maps (Cowardin et al., 1979; Ecological Stratification Working Group, 1995). However, wetlands can be difficult to work in largely due to overlapping habitat characteristics and naturally vague boundaries (U.S. Army Corps of Engineers, 2011) and inconsistencies in methodologies can lead to difficulty comparing wetlands between regions (Finlayson and van der Valk, 1995; Zedler and Kercher, 2005).

Forested wetlands are among the least studied wetlands (Sjöberg and Ericson, 1997). Due to inaccessibility, they are often overlooked or go undetected because of their tree canopy characteristics resembling those of an upland forest (Sader et al., 1995). Robust forested wetland species inventories are lacking for many forested wetland abundant organisms, included epiphytic lichens. Little is known about what lichens are found in forested wetlands and how they differ between wetland classes. Lichens are sensitive to smaller scale influences from their immediate surroundings. These composite organisms absorb everything through their cortex and cannot retain moisture due to the lack of a waxy cuticle, making lichens sensitive to air humidity, sun exposure, and substrate type (Purvis, 2000). Because of these characteristics, some lichens have been used as indicators of different habitats and air quality. For example, Kraichak et al. (2009) found moderate but significant differences in lichen composition between forest types and lichen associations with specific forested habitats on an Atlantic boreal island.

Indicator species can also be used to help with wetland classification and delineation (Tiner, 2017). Indicator species are organisms whose presence reflects the conditions of the environment where they occur (Siddig et al., 2016); wetland indicators reflect natural wetland processes and provide evidence of functioning wetlands (Tiner, 2017). Identifying individual species or suites of wetland indicators can facilitate wetland classification and delineation (Gage and Cooper, 2010). Lichvar et al. (2009) found bryophytes to be stronger wetland indicators than lichens because lichens were too sparsely distributed on the ground for strong wetland fidelity. However, I could not find any studies that investigated epiphytes for helping distinguish different forested wetland classes, specifically using epiphytic lichens on trees. Because trees are a distinct feature of these wetlands and arboreal lichens are a large component of many forested wetlands, arboreal macrolichens (i.e., foliose and fruticose growth forms), could be used to distinguish different forested wetland classes.

With 18% wetland cover (Zoltai and Pollett, 1983) and globally recognized as a hotspot for lichens (Ahti, 1983), Newfoundland is an ideal location to study macrolichens in forested wetlands. Many lichen surveys have been conducted in the province (McCarthy et al., 2015; McMullin and Arsenault, 2016; McMullin and Wiersma, 2017), and multiple studies have focused on rare macrolichens (Bowering et al., 2018; Goudie et al., 2011; Jørgensen et al., 2009). Although studies have investigated lichen ecology, none have focused specifically on lichens in forested wetlands. Therefore, a knowledge gap exists of what macrolichens are present in forested wetlands, how they are distributed between wetland classes and across landscapes (ecoregions), and if there are any indicator lichen species associated with forested wetlands.

The overall goal of my research was to investigate macrolichen ecology in forested wetlands on the island of Newfoundland. Specifically, I aimed to 1) compare whether and how macrolichen communities vary between forested wetland classes (bogs, fens, swamps) and geographic regions, and 2) detect if there are any macrolichens significantly associated with certain forested wetland classes or ecoregions that can be used as potential indicator species. I hypothesized that macrolichen community composition would be more different among forested wetland classes than ecoregions because of the characteristic affinity's lichens can have to specific habitats. If this hypothesis is supported, there would be a greater influence of forested wetland class than ecoregion on the macrolichen community composition. I also predicted that macrolichens would be associated with specific forested wetland classes and ecoregions and would be able to identify possible indicator species.

2.3 Methods

2.3.1 Study Areas

I conducted this study on the island of Newfoundland, Canada in the Avalon Forest, Central Newfoundland, and Northern Peninsula Ecoregions from May to August 2017 (Figure 2-1; Appendix 1 and 2). Within these ecoregions, I selected forested wetland sites within four areas of interest, one in the Avalon Forest, two in the Central Newfoundland and one in the Northern Peninsula ecoregions. These areas were originally selected by a wetland mapping project to which I contributed ground-truthing wetland classification data (Mahdavi et al., 2017). Once in each wetland mapping area of interest, I searched for forested wetland sites that met my criteria (see *Site Selection* below).

The Avalon Forest Ecoregion in eastern Newfoundland has a mean annual temperature of 5.5°C, mean annual precipitation of 1400-1500 mm and a forest dominated by balsam fir (*Abies balsamea*) <12 m tall, with black spruce (*Picea mariana*). The Central Newfoundland Ecoregion in north-central Newfoundland has a mean annual temperature of 3.0°C, mean annual precipitation of 1000-1300 mm, and a black spruce and balsam fir-dominated forest with paper birch (*Betula papyrifera*) and aspen (*Populus tremuloides*) in disturbed sites. The Northern Peninsula Ecoregion in western Newfoundland has a mean annual temperature of 5.5°C, mean annual precipitation of 1000-1100 mm, and a dominant balsam fir with co-dominant black spruce forest (Damman, 1983).

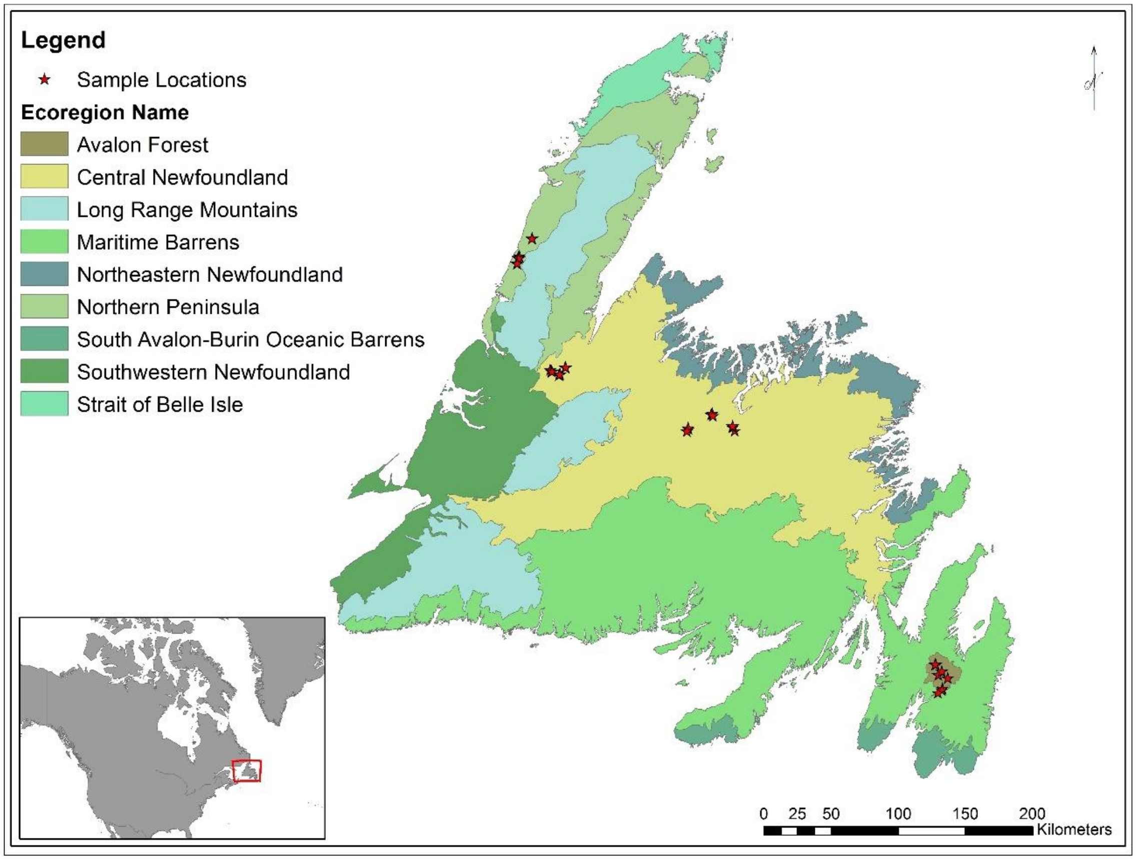

Figure 2-1. Map of the forested wetland (bog, fen, swamp) sites showing the ecoregions of Newfoundland. There were nine forested wetland sites in the Northern Peninsula Ecoregion, eighteen sites in the Central Newfoundland Ecoregion, and nine sites in the Avalon Forest Ecoregion.

2.3.2 Site Selection

I selected forested wetland sites by conducting reconnaissance surveys before starting fieldwork. The sites were broken down into three distinct classes of forested wetlands, which were selected using the Canada Wetland Classification System (National Wetlands Working Group, 1997) and Sjöberg and Ericson's (1997) forested wetland criteria. Photos of representative sites are in Appendix 3. The forested wetland classes are:

- Forested Bog – Moderate (~50%) canopy cover, rainwater as the primary water influence, ericaceous shrubs present and sphagnum moss dominant (>75%) ground cover. The plots for forested bogs were located in the forest surrounding a treeless bog <15 m from the bog opening.
- Forested Fen – Open (<25%) canopy cover due to sparse tree vegetation, water table is at or above the ground surface, graminoid dominant ground cover (>75%).
- Forested Swamp – Closed (>75%) canopy cover, water table is at or below the ground surface, sparse shrub cover, and shade tolerant moss ground cover (>75%).

2.3.3 Study Design

Within each of the four study areas, I selected nine sites (three bogs, three fens, three swamps), and set up a 5 m radius plot within each site in a representative location within the wetland. Within each plot, I conducted a forested wetland survey to characterize the wetland and a macrolichen survey on three live black spruce trees. Trees were haphazardly selected among those that fit the following selection criteria: >5 cm diameter at breast height (DBH), not leaning, and minimal branching or bark damage in the macrolichen survey area (0.9-1.9 m height above the ground). Black spruce trees were used because they were the dominant tree species in the wetlands and present in each ecoregion. In total, there were 36 plots (9 in the Avalon Forest and Northern Peninsula Ecoregions and 18 in the Central Newfoundland Ecoregion) and 108 trees surveyed.

For the forested wetland survey, I measured canopy closure, noted tree species present and stem count within the sample plots, conducted percent shrub and ground cover, and sampled soil pH. I measured canopy closure at the plot centre using a spherical densiometer (Forest Densiometers, model A). I visually estimated percent shrub cover vertically between 10-100 cm using a 2x2 m quadrat and percent ground cover vertically between 0-10 cm using a 1x1 m quadrat each placed at the plot center. I used the mean of three measurements taken at the plot centre (within the 1x1 m quadrat) with an ExStik® waterproof pH meter as an estimate of soil pH.

For the macrolichen survey, I selected three live black spruce trees and measured the DBH at 1.3 m and visually estimated tree height. I then surveyed for macrolichens between 0.9-1.9 m on the tree trunk by recording each macrolichen species and number of thalli as an estimate of abundance. I did not survey below 0.9 m on the tree bole to avoid sampling terricolous lichens. Percent cover was not estimated due to the characteristically dense overlapping of macrolichens (Kraichak et al., 2009). I identified most of the macrolichens in the field but collected difficult specimens for microscopic and chemotaxonomic examination in the laboratory. Specimen identification followed procedures in Brodo (2016), Brodo et al. (2001) and Hinds and Hinds (2007).

2.3.4 Data Analysis

All analyses were conducted using R statistical software (R version 3.5.2, R Core Team, 2018). Canopy cover and soil pH were compared among forested wetland classes using analysis of variance (ANOVA) with a significance level of $\alpha = 0.05$. I averaged the macrolichen species abundance data from the three surveyed trees within each plot to represent the plot. Macrolichen diversity was then calculated using the vegan package (Oksanen et al., 2017) and the Shannon Diversity Index (Shannon, 1948). I chose to use the Shannon Diversity Index because it is thought to more effectively quantify diversity in landscapes with rare habitat types (Nagendra, 2002). I used Kruskal-Wallis tests to assess statistical significance ($p \leq 0.05$) of differences among the diversity medians from the three ecoregions and the three forested wetland classes. The use of this non-parametric test was based on the lichen response data residuals not meeting the assumption of normality.

I performed a non-metric multidimensional scaling (NMDS) analysis to use an unconstrained exploratory method of visualizing the variation in macrolichen composition and the relationships with explanatory variables. NMDS is a robust and effective exploratory method for multivariate data and calculates a final configuration that preserves rank-order dissimilarities in the final reduced-dimension site matrix that are as close as possible to those in the original site matrix (McCune and Grace, 2002; Peck, 2016). I computed the NMDS analysis with the vegan package (Oksanen et al., 2017) using a square-root transformed distance matrix with the Bray-Curtis distance measure. I determined that three dimensions would be optimal based on the stress level. Stress is the value representing the difference between distance in the reduced dimension compared to the complete multidimensional space and a stress level between 0.1 and 0.2 is suitable, with a lower stress level being more desirable. An ordination of the final site scores was plotted to visualize how much overlap occurred among the different macrolichen communities. Ellipses representing 95% confidence intervals were used around each centroid of each forested wetland class and ecoregion to visualize the distinction of each group's composition. Explanatory site variable vectors were then fitted to the ordination plots to visualize explanatory variable influence using the vegan package (Oksanen et al., 2017). The vector arrows position indicates the direction of the gradient and the length indicates the strength of the gradient.

To test for the effect of ecoregion and forested wetland class on macrolichen composition, I used a non-parametric multivariate analysis of variance (perMANOVA). The decision to use perMANOVA was based on the lichen response residuals not meeting the assumption of normality, due to many zeros in the data matrix, and not being independent, due to species clumping assemblages (Anderson, 2001). The perMANOVA was computed using the vegan package (Oksanen et al., 2017) with a square-root transformed distance matrix using the Bray-Curtis distance measure (999 permutations) with a significance level of $\alpha = 0.05$. Before running the analysis, the macrolichen matrix and site database were modified to be balanced where the two Central Newfoundland clusters of sites were averaged to represent the entire ecoregion. I ran a full model with the two main fixed effects, ecoregion and forested wetland class, to determine if the interaction term was significant.

To determine if individual lichen species differed in their strength of association with wetland classes or ecoregions, I used an Indicator Species Analysis (ISA). Each lichen species was assigned an Indicator Value (IV), which represents the likelihood of finding it in each forested wetland class or ecoregion. To test the significance of observed indicator values ($\alpha = 0.05$), 1000 randomizations were iterated. Indicator Values range from no indication (zero) to perfect indication (100), and perfect indicators are always present and exclusive to a group. I conducted the Indicator Species Analysis using the labdsv package (Roberts, 2016) with Dufrêne and Legendre's (1997) method and used the Indicator Value threshold of >25 with a significant *p*-value for indicator species.

2.4 Results

The three distinct forested wetland classes (bogs, fens, swamps) showed significant differences in soil pH and canopy closure as well as different combinations of dominant ground and shrub cover (Table 2-1). The total number of macrolichen species found across all sites was 20 in 13 genera (Table 2-2). The most abundant macrolichen species overall (in all forested wetland classes, from highest to lowest) were *Bryoria* spp., *Alectoria sarmentosa, Hypogymnia physodes, Platismatia glauca,* and *Imshaugia aleurites*. However, lichen abundance varied among forested wetland classes. For example, *Imshaugia aleurites* was the fifth most common lichen found in all forested wetland classes, but it had a relative abundance of 43% in bogs, 51%, in fens and 6% in swamps. The highest macrolichen diversity was in the Northern Peninsula Ecoregion and in forested fens (Figure 2-2a, b).

Table 2-1. Characteristics of the three forested wetland classes (bog, fen, swamp) including soil pH, canopy closure, the dominant tree species in the plot, and the dominant vegetation in the shrub and ground layer. ANOVAs were used to compare means with the significance set at α = 0.05.

Characteristics	Forested Wetland Classes			*p*-values
	Bog	Fen	Swamp	
Soil pH (±SE)	3.57 (±0.07)	5.57 (±0.30)	4.08 (±0.17)	<0.001
Canopy closure (%)	46 (moderate)	21 (open)	77 (closed)	<0.001
Dominant tree species	Black spruce	Black spruce	Black spruce/balsam fir	-
Shrub layer	Ericaceous	Graminoids	Graminoids (sparse)	-
Ground layer	Sphagnum moss	Sphagnum moss	Sphagnum/feather moss	-

Table 2-2. Macrolichen thalli count by forested wetland class and ecoregion with relative abundance (%) in brackets.

Lichen Species	Forested Wetland			Ecoregion		
	Bog	Fen	Swamp	Avalon	Central	Northern
Alectoria sarmentosa	260 (50)	134 (26)	121 (24)	138 (40)	343 (50)	35 (10)
Bryoria spp.	350 (37)	335 (35)	264 (28)	414 (54)	366 (24)	167 (22)
Cladonia spp.	15 (20)	25 (34)	34 (46)	44 (64)	7 (5)	21 (31)
Coccocarpia palmicola	0	0	2 (100)	2 (100)	0	0
Evernia mesomorpha	0	2 (63)	1 (37)	0	0	3 (100)
Hypogymnia hultenii	2 (100)	0	0	0	0	2 (100)
H. incurvoides	15 (31)	13 (28)	19 (41)	17 (42)	12 (15)	18 (43)
H. physodes	105 (19)	200 (37)	242 (44)	118 (31)	330 (43)	100 (26)
H. tubulosa	41 (29)	46 (33)	53 (38)	52 (48)	64 (30)	24 (22)
H. vittata	48 (37)	36 (28)	46 (35)	7 (9)	102 (65)	20 (26)
Imshaugia aleurites	207 (43)	247 (51)	31 (6)	6 (2)	311 (47)	167 (51)
Parmelia squarrosa	29 (15)	111 (56)	58 (29)	77 (42)	28 (8)	94 (50)
P. sulcata	3 (15)	11 (51)	7 (34)	0	17 (67)	4 (33)
Parmeliopsis capitata	80 (75)	18 (17)	9 (8)	0	37 (21)	70 (79)
Platismatia glauca	133 (21)	234 (39)	240 (40)	139 (32)	331 (37)	137 (31)
P. norvegica	11 (34)	10 (32)	11 (34)	18 (54)	0	15 (46)
Sphaerophorus globosus	47 (53)	18 (20)	24 (27)	90 (100)	0	0
Usnea longissima	9 (20)	30 (69)	5 (11)	26 (73)	14 (19)	3 (8)
Usnea spp.	0	0	1 (100)	0	1 (100)	0
Vulpicida pinastri	8 (100)	0	0	0	0	8 (100)

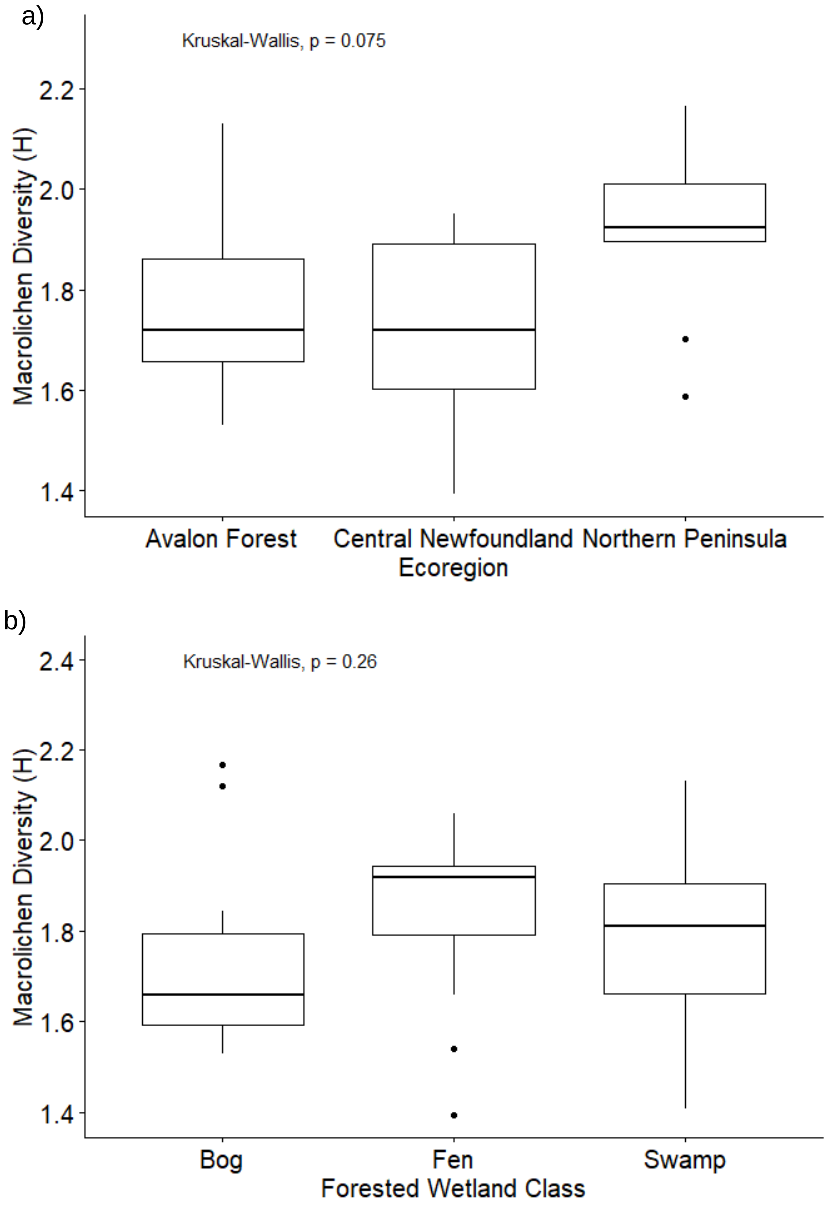

Figure 2-2. Boxplots comparing Shannon Diversity Index for lichens by a) ecoregion and b) forested wetland class. There were nine study sites in the Avalon Forest, 18 sites in Central Newfoundland, and nine in the Northern Peninsula. Kruskal-Wallis tests compared the differences in richness and diversity among ecoregions and forested wetland classes. Boxes represent 25th and 75th quartiles, and whiskers represent the minimum and maximum data points, exclusive of outliers.

In the NMDS analysis and plot, there was more overlap among the different forested wetland classes than ecoregions (Figure 2-3a, b). A final stress of 0.117 for a three-dimensional solution was obtained after 100 iterations using a random starting seed. Environmental variables were fitted as vectors to the ordination plot (Table 2-3) where tree height and DBH separated the Central Newfoundland Ecoregion sites from the Northern Peninsula and the Avalon Forest Ecoregions.

The results of the perMANOVA showed there to be a significant difference in macrolichen community composition among the three ecoregions ($p = 0.001$) and among the three forested wetland classes ($p = 0.002$). The interaction term for ecoregion and forested wetland class was not significantly related (Table 2-4).

Based on the Indicator Species Analysis, multiple macrolichen species were selected as potential indicator species for both forested wetland classes and ecoregions. This indicator species designation means that the selected lichens were more likely to be found in certain forested wetlands or ecoregions (Table 2-5). Two macrolichen species, *Alectoria sarmentosa* (IV 50.5) and *Usnea longissima* (IV 46.4), were significantly associated with forested bogs and forested fens, respectively. Seven macrolichen species were significantly associated with different ecoregions. Macrolichens significantly associated with the Avalon Forest Ecoregion were *Bryoria* spp. (IV 54.2), *Cladonia* spp. (IV 49.8), *Sphaerophorus globosus* (IV 88.9), and *Usnea longissima* (IV 64.5). Macrolichens significantly associated with the Northern Peninsula Ecoregion were *Hypogymnia incurvoides* (IV 43.2) and *Vulpicida pinastri* (IV 33.3), and with the Central Newfoundland Ecoregion was *H. vittata* (IV 64.5) (Monte Carlo Test, $p < 0.05$).

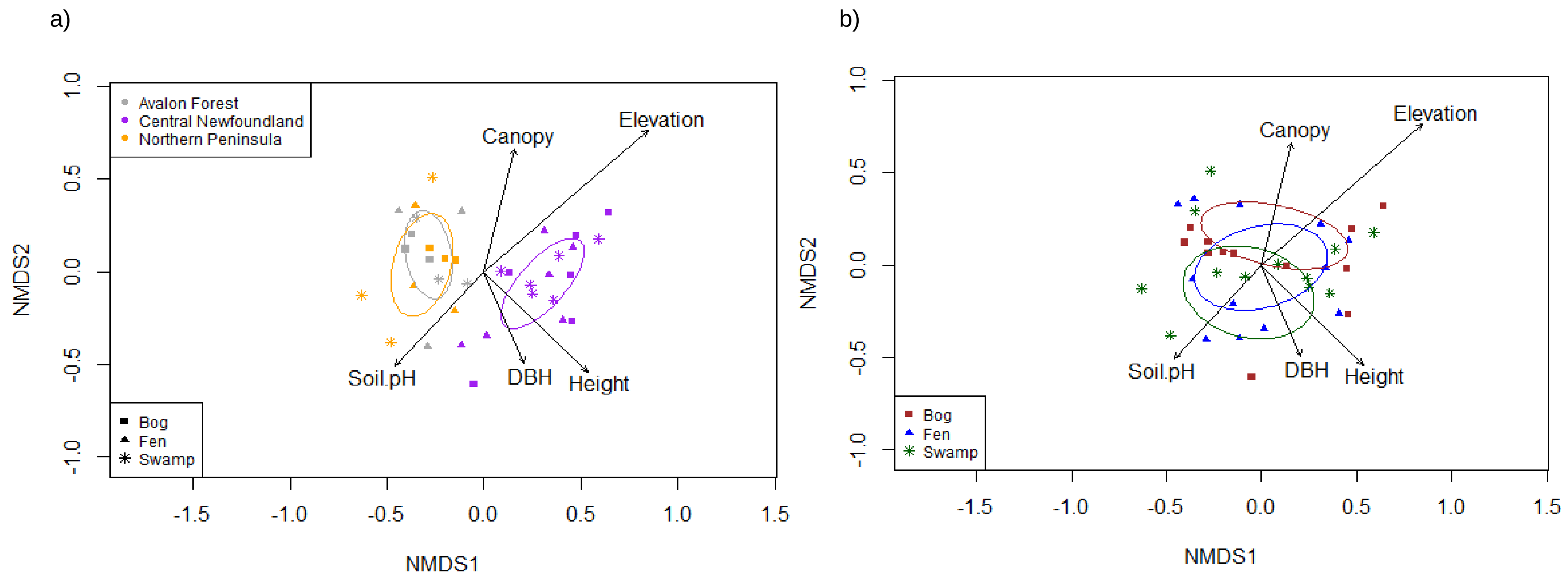

Figure 2-3. Ordination results of the non-metric multidimensional scaling (NMDS) analysis with a) ecoregions and b) forested wetland classes. A final stress of 0.117 for a three-dimensional solution was concluded. Each data point represents a site and ellipses represent 95% confidence intervals around each centroid. Environmental site variables were fitted as vectors with the vector arrows position indicating the direction of the gradient and length indicating the strength of the gradient.

Table 2-3. Explanatory variable vectors fitted to the non-metric multidimensional (NMDS) scaling analysis plot. The number of permutations was 999 and the significance level was α = 0.05. The NMDS 1 and 2 columns give the cosines of the vectors, and the R^2 values give the squared correlation coefficient. DBH = diameter at breast height.

Variable	NMDS 1	NMDS 2	R^2	*P* (>r)
Canopy cover	0.23346	0.97237	0.0937	0.200
Soil pH	-0.67234	-0.74025	0.0951	0.192
Elevation	0.74192	0.67049	0.2626	0.008
Tree DBH	0.38734	-0.92194	0.0585	0.367
Tree height	0.70388	-0.71032	0.1167	0.128

Table 2-4. Results from the non-parametric multivariate analysis of variance (perMANOVA) using Bray-Curtis distance with 999 permutations and a square-root transformed distance matrix. Data were modified to have a balanced design by averaging the Central Newfoundland sites together and significance was α = 0.05.

Source	df	SS	MS	F	R^2	*p-value*
Ecoregion	2	0.70998	0.35499	9.7357	0.39521	0.001
Forested Wetland Class	2	0.25352	0.12676	3.4764	0.14112	0.002
Interaction Term	4	0.17663	0.04416	1.2110	0.09832	0.256
Residuals	18	0.65633	0.03646	-	0.36535	-
Total	26	1.79646	-	-	1.00000	-

Table 2-5. Significant associations of macrolichens with forested wetlands and ecoregions (maximum habitat association); the observed Indicator Values show the strength of the association, along with a *p*-value (α = 0.05).

Lichen Species	Forested Wetland		
	Maximum Habitat Association	Observed Indicator Value	*p*-value
Alectoria sarmentosa	Bog	50.5	0.0244
Usnea longissima	Fen	46.4	0.0212
	Ecoregion		
	Maximum Habitat Association	Observed Indicator Value	*p*-value
Bryoria spp.	Avalon	54.2	0.0008
Cladonia spp.	Avalon	49.8	0.0192
Hypogymnia incurvoides	Northern	43.2	0.0462
H. vittata	Central	65.5	0.0002
Sphaerophorus globosus	Avalon	88.9	0.0002
Usnea longissima	Avalon	64.5	0.0012
Vulpicida pinastri	Northern	33.3	0.0254

2.5 Discussion

I found differences in macrolichen community composition, but with less distinction between communities in forested wetland classes than communities in ecoregions. Ecoregion (i.e., the geographical scale) had more of an influence on forested wetland macrolichen diversity and community composition. I identified potential macrolichen indicator species for forested wetland classes and each ecoregion.

I found that macrolichen diversity was highest in the Northern Peninsula Ecoregion and in forested fens. Gros Morne National Park is recognized for its rich diversity of vegetation representing over half of the island of Newfoundland's total flora and with a range of habitats compared to the rest of the island (Bouchard et al., 1991). It seems likely that the high lichen diversity I observed in this region is at least in part related to these features. Forested fens are a sparsely treed, open canopy habitat with high sun and wind exposure. The greater available sunlight would enhance photosynthesis of the photobiont symbiotes and high wind exposure would increase spore or fragment wind dispersal (Brodo et al., 2001), potentially promoting higher lichen diversity at these sites (Moning et al., 2009; Uliczka and Angelstam, 1999).

Macrolichen communities differed among ecoregions and forested wetland classes, with more notable separation of ecoregion lichen communities in the ordination plot. The Central Newfoundland Ecoregion macrolichen communities were distinctly separated in the NMDS ordination plot from the other ecoregions and the Avalon Forest and Northern Peninsula Ecoregions have some overlap, even though they are the farthest apart at approximately 450 km apart with ~3° latitude change (Gros Morne 50° N and Avalon 47° N). This pattern is likely due to the inland location of the Central

Newfoundland sites in contrast to the more coastal locations of the Northern Peninsula and Avalon Forest sites. The coastal sites in the Avalon Forest and Northern Peninsula are more exposed to fog and humid weather with less extreme temperature ranges, while the Central Newfoundland sites are subject to lower air humidity and more extreme temperature ranges. Both *Cladonia* spp. and *Platismatia norvegica* showed preference to the coastal areas as they were found mainly in the Northern Peninsula and Avalon Forest Ecoregions with low abundance or no detection in the Central Newfoundland Ecoregion. Another lichen worth noting for coastal habitat preference, though I did not detect it on my surveyed tree but did detect it in the sites, is *Erioderma pedicellatum.* This Species-at-Risk lichen is only found close to the coast where the humidity levels are high from fog, and it is not known to occur in the interior of the island of Newfoundland (Maass and Yetman, 2002; Wiersma and Skinner, 2011).

The lack of separation between macrolichen communities in different forested wetland classes could have resulted from focusing only on macrolichens and not including microlichens in the lichen surveys. Microlichens (crustose growth forms along with calicioids) have been shown to be more sensitive to different habitats (Bunnell et al., 2008; McMullin and Arsenault, 2016). Including these in my surveys could have allowed greater detection of differences between the forested bogs, fens, and swamps. Similarly, not identifying all the fruticose lichens to species level may have also reduced my ability to discriminate among wetland types. Moreover, certain lichens are better indicators for larger extents (regions) versus smaller extents (habitats). Common lichens are often used to detect pollutions levels covering large areas (McMullin et al., 2017; Wadleigh, 2003) and may be better for identifying regional differences. The majority of the macrolichens I detected in our surveys were relatively common species. To detect differences in the different forested wetland classes, microlichens or less common lichens may be better indicators of habitat differences. However, these are more difficult to identify and require more specialized skill and knowledge.

The best indicator species was *Sphaerophorus globosus*, which was strongly associated with the Avalon Forest Ecoregion forested wetlands. However, this lichen is quite common on the Avalon Peninsula generally and is not necessarily specific to forested wetlands (McCarthy et al., 2015; McMullin and Wiersma, 2017). The Avalon Forest is an ideal habitat for *S. globosus* due to high relative air humidity from coastal fog. Other lichens associated with the Avalon Forest Ecoregion were *Bryoria* spp. and *Cladonia* spp. which also have an affinity for high relative humidity. Lichen species *Alectoria sarmentosa* was associated with forested bogs, however, it is abundant throughout Newfoundland forests (Wiseman and Wadleigh, 2002) so would not be useful as a bog indicator more generally. In the Central Newfoundland Ecoregion, *Hypogymnia vittata* was found to be significantly associated with the ecoregion, which might have to do with an affinity to inland habitat. One lichen, *Usnea longissima*, was significantly associated with forested fens and the Avalon Forest Ecoregion. In this study, *U. longissima* preferred open canopy habitats which contradicts the findings of Esseen et al. (1981) in that *U. longissima* showed a strong preference for sites protected from the wind, in more closed canopy settings. The lichen species *Hypogymnia incurvoides* and *Vulpicida pinastri* were associated with the Northern Peninsula Forest Ecoregion. A common lichen found in bogs, *V. pinastri* was quite abundant across the island but usually grew low to the ground and did not make it in the survey area of 0.9-1.9 m on the tree bole. However, in the Northern Peninsula, *V. pinastri* grew higher on the tree and was included in the survey area. I regularly observed all the macrolichen indicator species described above in non-wetland forests.

Unusual occurrences of certain lichen species detected in this study require further comment. The presence of *Parmelia sulcata* in some of the western sites in the Central Newfoundland Ecoregion may have been due to surveying in an agricultural area that could have higher air pollution levels. Some studies have found *P. sulcata* present in areas of moderate air pollution making it a potential indicator for certain polluted areas (Monaghan and Wiersma, 2018; Will-Wolf et al., 2006). The presence of *Cladonia* spp., which is typically a ground lichen, was largely restricted to situations where the tree being sampled was slightly leaning or had a burl present that created a moist growing platform. The only cyanolichen detected in this study was *Coccocarpia palmicola* in a forested swamp in the Avalon Forest. As a known boreal felt lichen (*E. pedicellatum*) indicator (Cornejo and Scheidegger, 2016), *C. palmicola* was detected in an area with many *E. pedicellatum*, however, no *E. pedicellatum* were detected on the black spruce trees I surveyed for the study most likely due to its known affinity for balsam fir in the Avalon Forest (Wiersma and Skinner, 2011).

While surveying lichens in forested wetlands was the purpose of this study, not including non-wetland forests did limit the scope of finding wetland indicator species. This decreased the robustness of the Indicator Species Analysis by not verifying if the lichen associations were specific to only wetlands and not also found in non-wetland forests. A potential source of bias in this study was only focusing on macrolichens and excluding microlichens in the surveys. This could have led to missing some of the subtler differences between habitats, sometimes detected with microlichen surveys. However, not identifying the *Bryoria* and *Usnea* spp. to species level may have been a larger limitation because most of the indicator species were these lichens and not identifying them to species would decrease specificity within the genera.

Future studies should include macrolichen surveys in non-wetland forest habitats so that the specificity of lichen species for forested wetlands can be determined. The results of this study will contribute to increasing the knowledge and understanding of macrolichen community composition and forested wetland ecology.

2.6 References

Ahti, T. (1983). Lichens. In G. R. South (Ed.), Biogeography and Ecology of the Island of Newfoundland (pp. 319–360). The Hague, the Netherlands: Dr. W Junk Publishers.

Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. Austral Ecology 26: 32–46.

Barbier, E. B., Acreman, M., and Knowler, D. (1997). Economic valuation of wetlands: A guide for policy makers and planners. Gland, Switzerland: Ramsar Convention Bureau.

Bouchard, A., Hay, S., Bergeron, Y., and Leduc, A. (1991). The vascular flora of Gros Morne National Park, Newfoundland: A habitat classification approach based on floristic, biogeographical and life-form data. In Quantitative approaches to phytogeography (pp. 183–204). Dordrecht: Springer.

Bowering, R., Wigle, R., Padgett, T., Adams, B., Cote, D., and Wiersma, Y. F. (2018). Searching for rare species: A comparison of Floristic Habitat Sampling and Adaptive Cluster Sampling for detecting and estimating abundance. Forest Ecology and Management 407: 1–8.

Brodo, I. M. (2016). Keys to Lichens of North America. New Haven and London: Yale University Press.

Brodo, I. M., Sharnoff, S. D., and Sharnoff, S. (2001). Lichens of North America. New Haven and London: Yale University Press.

Bunnell, F. L., Spribille, T., Houde, I., Goward, T., and Björk, C. R. (2008). Lichens on down wood in logged and unlogged forest stands. Canadian Journal of Forest Research 38: 1033–1041.

Cornejo, C. and Scheidegger, C. (2016). Cyanobacterial gardens: The liverwort *Frullania asagrayana* acts as a reservoir of lichen photobionts. Environmental Microbiology Reports 8: 352–357.

Cowardin, L. M., Carter, V., Golet, F. C., and LaRoe, E. T. (1979). Classification of wetlands and deepwater habitats of the United States. FGDC-STD-004-2013. Second Edition 79.

Damman, A. W. H. (1983). An ecological subdivision of the island of Newfoundland. In G. R. South (Ed.), Biogeography and Ecology of the Island of Newfoundland (pp. 163–206). The Hague, the Netherlands: Dr. W Junk Publishers.

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

Ecological Stratification Working Group (1995). A national ecological framework for Canada. Centre for Land and Biological Resources, research branch Agriculture and Agri-Food Canada. State of the Environment Directorate, Environment Conservation Service, Environment Canada.

Esseen, P. A., Ericson, L., Lindstrom, H., and Zackrisson, O. (1981). Occurrence and ecology of *Usnea longissima* in central Sweden. The Lichenologist 13: 177–190.

Finlayson, C. M. and van der Valk, A. G. (1995). Wetland classification and inventory: A summary. Vegetatio 118: 185–192.

Gage, E. and Cooper, D. J. (2010). Vegetation sampling for wetland delineation: A review and synthesis of methods and sampling issues. U.S. Army Corps of Engineers Cold Regions Research and Engineering Laboratory, Washington, DC ERDC/CRREL CR-10-2.

Goudie, R. I., Scheidegger, C., Hanel, C., Munier, A., and Conway, E. (2011). New population models help explain declines in the globally rare boreal felt lichen *Erioderma pedicellatum* in Newfoundland. Endangered Species Research 13: 181–189.

Hinds, J. W., and Hinds, P. L. (2007). Macrolichens of New England. Bronx, NY: New York Botanical Garden Press.

Jørgensen, P. M., Clayden, S. R., Hanel, C., and Elix, J. A. (2009). *Erioderma mollissimum* (Pannariaceae) found with certainty in Newfoundland, Canada. The Bryologist 112: 572–575.

Kraichak, E., Pope, R., and Wheelwright, N. T. (2009). Habitat associations of macrolichens on a boreal island in the Bay of Fundy, New Brunswick, Canada. The Bryologist 112: 762–772.

Maass, W. and Yetman, D. J. (2002). COSEWIC assessment and status report on the boreal felt lichen *Erioderma pedicellatum*. COSEWIC Assessment and Status Report.

Mahdavi, S., Salehi, B., Amani, M., Granger, J. E., Brisco, B., Huang, W., and Hanson, A. (2017). Object-based classification of wetlands in Newfoundland and Labrador using multi-temporal PolSAR data. Canadian Journal of Remote Sensing 43: 432–450.

McCarthy, J., Driscoll, K., and Clayden, S. R. (2015). Lichens in four Newfoundland provincial parks: New provincial records. The Canadian Field-Naturalist 129: 219–228.

McCune, B. and Grace, J. B. (2002). Analysis of ecological communities (3rd ed.). Gleneden Beach, OR: MjM Software Design.

McMullin, R. T. and Arsenault, A. (2016). The calicioids of Newfoundland, Canada. Opuscula Philolichenum, 15: 92–104.

McMullin, R. T., Ure, D., Smith, M., Clapp, H., and Wiersma, Y. F. (2017). Ten years of monitoring air quality and ecological integrity using field-identifiable lichens at Kejimkujik National Park and National Historic Site in Nova Scotia, Canada. Ecological Indicators 81: 214–221.

McMullin, R. T. and Wiersma, Y. F. (2017). Lichens and allied fungi of Salmonier Nature Park, Newfoundland. The Journal of the Torrey Botanical Society 144: 357–369.

Monaghan, M., and Wiersma, Y. F. (2018). *Parmelia sulcata* as a bioindicator of air pollution in Newfoundland, Canada. Evansia 35: 30–35.

Moning, C., Werth, S., Dziock, F., Bässler, C., Bradtka, J., Hothorn, T., and Müller, J. (2009). Lichen diversity in temperate montane forests is influenced by forest structure more than climate. Forest Ecology and Management 258: 745–751.

Nagendra, H. (2002). Opposite trends in response for the Shannon and Simpson indices of landscape diversity. Applied Geography 22: 175–186.

National Wetlands Working Group (1997). The Canadian wetland classification system. Waterloo, Canada: Wetlands Research Branch, University of Waterloo.

Oksanen, J., Guillaume Blanchet, F., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., … Wagner, H. (2017). Vegan: Community ecology package. R package version 2.4-6.

Peck, J. E. (2016). Multivariate analysis for ecologists: Step-by-step (2nd ed.). Gleneden Beach, OR: MjM Software Design.

Purvis, W. (2000). Lichens. The Natural History Museum, London: Smithsonian Institution Press in association with the Natural History Museum, London.

R Core Team (2018). R: A language and environment for statistical computing. Vienna Austria: R Foundation for Statistical Computing.

Roberts, D. W. (2016). labdsv: Ordination and multivariate analysis for ecology. R package version 1.8-0.

Sader, S. A., Ahl, D., and Liou, W. S. (1995). Accuracy of landsat-TM and GIS rule-based methods for forest wetland classification in Maine. Remote Sensing of Environment 53: 133–144.

Shannon, C. E. (1948). A Mathematical Theory of Communication. The Bell System Technical Journal 27: 379–423.

Siddig, A. A. H., Ellison, A. M., Ochs, A., Villar-Leeman, C., and Lau, M. K. (2016). How do ecologists select and use indicator species to monitor ecological change? Insights from 14 years of publication in Ecological Indicators. Ecological Indicators 60: 223–230.

Sjöberg, K. and Ericson, L. (1997). Mosaic boreal landscapes with open and forested wetlands. Ecological Bulletins 46: 48–60.

Tiner, R. (2017). Wetland Indicators: A guide to wetland formation, identification, delineation, classification, and mapping (Second Edi). Boca Raton: Taylor & Francis.

U.S. Army Corps of Engineers (2011). Regional Supplement to the Corps of Engineers Wetland Delineation Manual: Northcentral and Northeast Region (version 2.0). Vicksburg, MS: U.S. Army Engineer Research and Development Center.

Uliczka, H. and Angelstam, P. (1999). Occurrence of epiphytic macrolichens in relation to tree species and age in managed boreal forest. Ecography 22: 396–405.

Wadleigh, M. A. (2003). Lichens and atmospheric sulphur: What stable isotopes reveal. Environmental Pollution 126: 345–351.

Wiersma, Y. F. and Skinner, R. (2011). Predictive distribution model for the boreal felt lichen *Erioderma pedicellatum* in Newfoundland, Canada. Endangered Species Research 15: 115–127.

Will-Wolf, S., Geiser, L. H., Neitlich, P., and Reis, A. H. (2006). Forest lichen communities and environment: How consistent are relationships across scales? Journal of Vegetation Science 17: 171–184.

Wiseman, R. D. and Wadleigh, M. A. (2002). Lichen response to changes in atmospheric sulphur: Isotopic evidence. Environmental Pollution 116: 235–241.

Zedler, J. B. and Kercher, S. (2005). Wetland Resources: Status, trends, ecosystem services, and restorability. Annual Review of Environment and Resources 30: 39–74.

Zoltai, S. C. and Pollett, F. C. (1983). Wetlands in Canada: Their classification, distribution, and use. In Ecosystems of the World 4B. Mires: Swamp, Bog, Fen and Moor. Regional Studies (pp. 245–268).

Chapter 3 Macrolichen richness, diversity, and composition in boreal forested swamps, ecotones, and upland forests

3.1 Abstract

Open wetlands have been well studied and are found to have higher biodiversity than non-wetland ecosystems. Forested wetlands and their biodiversity, however, are understudied and less understood. Epiphytes, such as lichens, are an abundant feature in forested wetlands and could be used to compare species richness and diversity between forested swamps, swamp-upland ecotones, and upland forests. I investigated the potential use of epiphytic macrolichens as a surveying tool in 15 study sites in the Avalon Forest Ecoregion, Newfoundland. Within each study site I set up three parallel 40 m transects in the 1) forested swamp, 2) ecotone, and 3) upland forest. Along each transect, I selected five balsam fir (*Abies balsamea*) trees about 10 m apart. I surveyed each tree for macrolichens on the bole and measured diameter at breast height and tree height. At each transect, I selected two trees of average size to core for age and measured canopy cover at the transect centre. I found that average macrolichen richness and diversity were highest in forested swamps, slightly lower at the ecotones, and lowest in upland forests. Macrolichen community composition significantly differed among swamps, ecotones, and upland forests. The results of this study suggest using lichens as a tool for measuring biodiversity and that forested wetlands are key areas of conservation.

3.2 Introduction

Forested wetlands provide key ecosystem services and support a diversity of flora and fauna, yet often their inconspicuous nature and inaccessibility means they are understudied (Flinn et al., 2008; Hornberg et al., 1998; Sjöberg and Ericson, 1997). Forested wetlands, and in particular swamps, often get included with adjacent forest ecosystems during forest surveys or inventory mapping efforts. This happens because of contiguous forest canopies between the forested wetland and surrounding forest (Townsend, 2001), particularly when forested wetlands are small. Small wetlands surrounded by non-wetland habitats often support distinct assemblages of species, including rare species that rely on wetland refuges (Flinn et al., 2008). Moreover, forested wetland and upland forest ecosystems are believed to be ecologically distinct. But because forested wetlands are understudied, exactly how forested wetlands differ from upland forests is largely unknown.

The ecotones between forested wetlands and adjacent areas of open wetland or closed upland forest, like all ecotones, are hypothesized to support more species due to the overlapping of two communities (Kark, 2013; Kark and van Rensburg, 2006). Vaisanen (1992) found the highest lepidopteran richness and abundance at the ecotone of a bog and forest, which was attributed to the shelter of the tree canopy and shrubs and a favourable microclimate. However, the opposite has been detected depending on the type of ecotone (e.g., hard/abrupt vs. soft/diffuse edge) and the study taxa. Few studies have looked at lichen diversity in ecotones, and of the studies that have, results have been mixed (Grytnes et al., 2006; Hauck et al., 2012; Kuusinen, 1996). Kuusinen (1996) compared lichen diversity at soft ecotones adjacent to swamps and uplands in a boreal forest system and did not find higher diversity within the ecotone.

Epiphytes, specifically arboreal lichens, are an abundant feature in forested swamps and provide important contributions to forest ecosystem functions (Ellis, 2012). Lichens are highly influenced by the environment they live in due to absorbing moisture and particles through their cortex (Purvis, 2000). Because of this characteristic, lichens can be used as indicators of the environment they live in (Kraichak et al., 2009) as well as for monitoring pollution (McMullin et al., 2017) and changes to ecosystems (Berglund, 2004). They are also among the most sensitive organisms to climate change in forest habitats (Nascimbene and Spitale, 2017). The responsiveness of lichens to humidity suggests forested wetlands maybe a “hotspot” for lichens in the landscape resulting in increased biodiversity levels. While high diversity of vascular plants is well-documented in wetland ecosystems (Flinn et al., 2008), non-vascular epiphytes are less studied and diversity patterns are unknown (Ellis, 2012).

The overall goal of this research was to compare macrolichen richness, diversity, and community composition in forested swamps, swamp-upland ecotones, and adjacent upland forests on the island of Newfoundland. I hypothesized that macrolichen richness and diversity would differ among swamps, ecotones, and upland forests and that 1) diversity would be higher in swamps due to the abundant moisture. Alternatively, I hypothesized that 2) ecotones might have the highest richness and diversity due to the overlap of two habitats, and consistent with ecotone patterns found in other taxa. Finally, I hypothesized that 3) macrolichen community composition of forested swamps and upland forests are distinct and overlap in ecotones. Insights gained from testing these hypotheses will help to understand the contributions that forested swamps make to broader boreal forest lichen diversity, and also help us understand whether and how forested swamps can be distinguished from upland forests.

3.3 Methods

3.3.1 Study Areas

I conducted this study on the island of Newfoundland, Canada in the Avalon Forest Ecoregion from May to August 2018. Within this ecoregion, I selected 15 replicate sites (Figure 3-1; Appendix 4). The Avalon Forest Ecoregion in eastern Newfoundland has a mean annual temperature of 5.5°C, mean annual precipitation range of 1400-1500 mm and a balsam fir (*Abies balsamea*) dominated forest under 12 m tall with black spruce (*Picea mariana*) present (Damman, 1983).

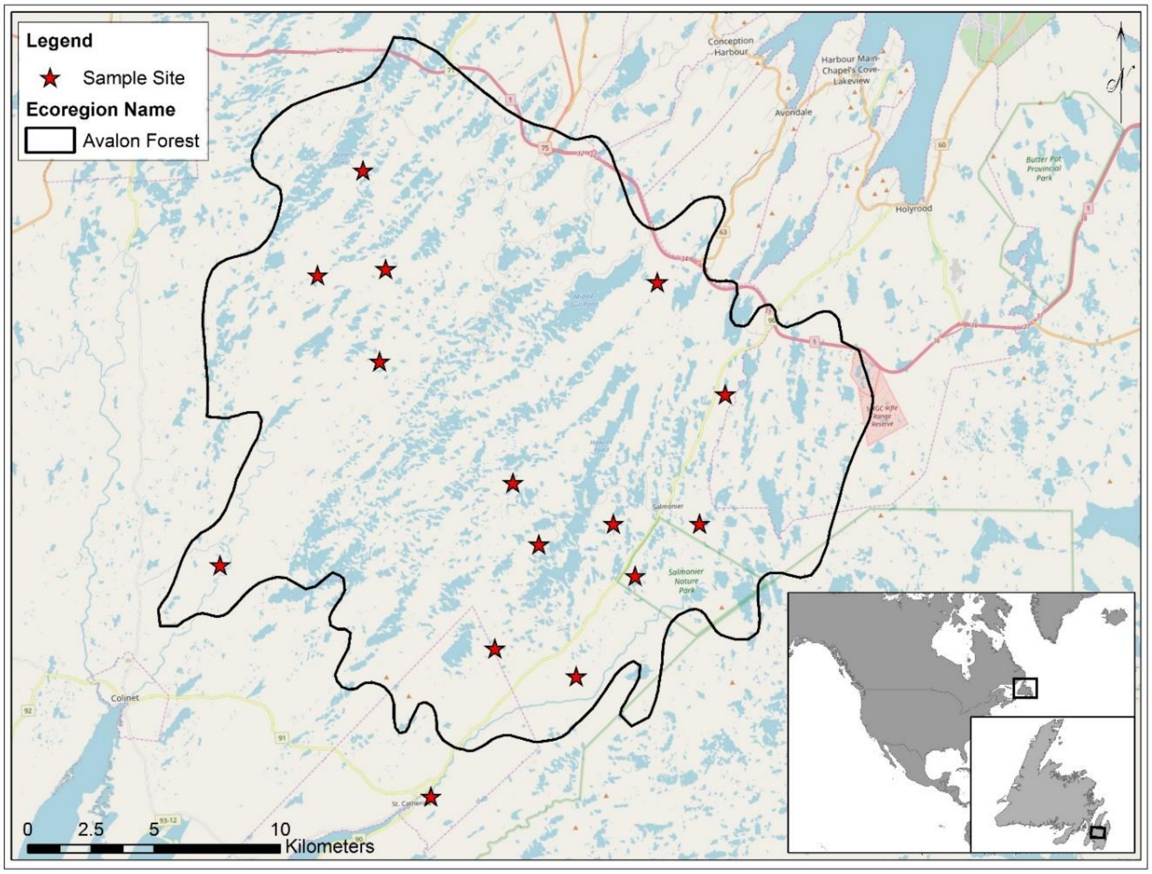

Figure 3-1. Map of study areas showing the 15 sites within the Avalon Forest Ecoregion. One site is just outside of the ecoregion boundary because of being on the private land of a partner on the project.

3.3.2 Site Selection

I selected the 15 sites using the Canada Wetland Classification System (National Wetlands Working Group, 1997) to identify the wetland portion of the site. Each site consisted of a forested swamp with an adjacent upland forest. Each forested swamp had closed canopy cover (~75% cover), water table at or below the ground surface, sparse shrub cover, and shade tolerant sphagnum and feather moss ground cover. Each upland forest had a closed canopy (~75% cover), sparse shrub layer, and feather moss ground cover with enough drainage so that soils do not become saturated for long periods such that no obligate hydrophytic plants or indicators of aquatic processes were present. I used the slope of the ground and the presence of sphagnum moss to aid in determining the ecotone between the swamp and upland forests. Specifically, I placed my ecotone survey locations where the topography began to rise, and the sphagnum/feather moss ratio was approximately 50:50 (Appendix 5).

3.3.3 Study Design

Within each of the study sites, I set up three 40 m transects: one in the forested swamp, one at the ecotone, and one in the upland forest. All the transects were positioned parallel to the ecotone and to each other and were placed approximately 30 m (20-40 m) apart depending on where the communities were best represented. Five live balsam fir trees were selected along each transect to survey for lichens; one at the ends of the transect and the others spaced approximately 10 m apart. Individual trees selected had at least 5 cm diameter at breast height (DBH, 1.3 m) and were not leaning more than 10°. If a tree at the desired position did not meet the criteria, I selected another up to 2 m in any direction. I used only the dominant balsam fir trees in order to have the same tree species in the swamp, ecotone, and upland. Along each transect, I conducted lichen tree surveys and a site survey.

I surveyed lichens on five live balsam fir trees along each transect. I measured the DBH of each tree with a DBH tape and calculated tree height using a clinometer. I then surveyed for lichens between 0.9-1.9 m (from the ground) on the tree bole and recorded each macrolichen species and number of each species' thalli. I did not survey under 0.9 m to avoid sampling terricolous lichens, and percent cover was not estimated due to characteristic dense overlapping of macrolichens (Kraichak et al., 2009). I identified most of the macrolichens in the field but collected difficult specimens for microscopic and chemotaxonomic examination in the laboratory. Specimen identification followed procedures outlined in Brodo (2016), Brodo et al. (2001) and Hinds and Hinds (2007).

Sites were also surveyed for canopy closure and tree age. I measured canopy closure at the centre point (20 m mark) of each transect using a spherical densiometer (Forest Densiometers, model A). I obtained tree age by selecting the average sized (by DBH) trees along the transects and using an increment borer to sample tree cores. Two trees per transect were cored with a total of 90 trees. The cores were taken as low down as possible on the tree bole (0.4-0.9 m above ground surface) to most accurately estimate the age. The cores were processed in the lab by gluing them on to grooved wood blocks and left to air dry. I then hand sanded them using the following sequence of sandpaper grit: 80 (coarse), 120, 220, 400 (fine). Once sanded, I counted the rings from the center to the bark, but not including the bark, using a 10x hand lens or dissecting microscope.

3.3.4 Data Analysis

All analyses were conducted using R statistical software (R version 3.5.2, R Core Team, 2018). Site characteristics were compared among transects using analysis of variance (ANOVA) with a significance level of α = 0.05. I averaged tree-level macrolichen species abundance data along each transect. Macrolichen richness and Shannon diversity were then calculated with the vegan package (Oksanen et al., 2017). I used Kruskal-Wallis tests to assess if the three transect groups (swamp, ecotone, upland) were significantly different, followed by pairwise comparisons to detect differences among transect types if the overall test was significant. All comparisons were set at a significance level of α = 0.05 and the use of this non-parametric test was based on the lichen response data residuals not meeting the assumption of normality.

To determine if individual lichen species differed in their frequency of occurrence among transect types, I used an Indicator Species Analysis (ISA). Each lichen species was assigned an indicator value (IV), which represents the likelihood of finding it in each group (i.e., swamp vs. ecotone vs. upland). Monte Carlo simulations (4999 permutations) were used to test the significance of observed indicator values (α = 0.05). Indicator values range from no indication (zero) to perfect indication (100), and perfect indicators are always present and exclusive to a group. I conducted the Indicator Species Analysis using the labdsv package (Roberts, 2016) with Dufrêne and Legendre’s (1997) method. Only indicators with an indicator value > 25 were considered significant regardless of the *p*-value associated with the result based on recommendations in Dufrêne and Legendre (1997).

I performed a non-metric multidimensional scaling (NMDS) analysis to use an unconstrained exploratory method of visualizing the variation in lichen composition and the relationship to environmental variables. NMDS is a robust and effective exploratory method for multivariate data, which tries to find a configuration in a given number of dimensions that preserves rank-order dissimilarities as closely as possible (McCune and Grace, 2002; Peck, 2016). I computed the NMDS analysis using the vegan package (Oksanen et al., 2017) with a double-root transformed distance matrix using the Bray-Curtis distance measure. I determined that three dimensions would be optimal based on the stress level. Stress is the value representing the difference between distance in the reduced dimension compared to the complete multidimensional space and a stress level between 0.1 and 0.2 is suitable, with a lower stress level being more desirable. An ordination of the scores was plotted to visualize how much overlap there was among the different lichen communities associated with each transect type. Ellipses representing 95% confidence intervals were used around each centroid of each transect type to visualize the distinction of each group's composition. Explanatory site variable vectors were then fitted to the ordination plots to visualize explanatory variable influence using the vegan package (Oksanen et al., 2017). The vector arrows position indicates the direction of the gradient and the length indicates the strength of the gradient.

To test for the effect of transect type on macrolichen composition, I used a non-parametric multivariate analysis of variance (perMANOVA). The decision to use perMANOVA was based on the lichen response data residuals not meeting the assumption of normality, due to many zeros, and not being independent, due to species clumping assemblages (Anderson, 2001). The perMANOVA was computed using the vegan package (Oksanen et al., 2017) and the Bray-Curtis distance measure with 999 permutations with a significance level of $\alpha = 0.05$.

3.4 Results

The site characteristics across transect types (swamp, ecotone, upland) were similar with respect to canopy cover ($p = 0.47$) and tree age ($p = 0.12$), but tree DBH and tree height differed significantly ($p < 0.05$). Trees were taller and larger in the upland forest than the swamp, but overall, forests along transect types were of similar age and canopy cover (Table 3-1). The total number of macrolichen species found across all sites was 22 in 16 genera (Table 3-2) with 21 species in the swamps, 18 species in the ecotones, and 16 species in the upland forests. Swamp transects had an average of 12.33 ± 0.37 macrolichen species, ecotone transects had 11.87 ± 0.68 species, and upland forest transects had 9.73 ± 0.62 species. Significant ($\alpha = 0.05$) species associations with specific transect types were detected with *Alectoria sarmentosa* (IV 61.2) and *Bryoria* spp. (IV 42.8) being associated with swamps.

Table 3-1. Characteristics of the three transect types (swamp, ecotone, upland) showing mean canopy closure, tree age, tree diameter at breast height (DBH), and tree height. ANOVAs were used to compare means with the significance set at α = 0.05. Each transect has an n = 15 except for tree age (n = 30) as two trees were cored per transect. Values shown are mean (±SE).

Characteristics	Swamp	Ecotone	Upland	*p*-value
Canopy closure (%)	86.24 (±0.85)	86.83 (±1.63)	88.32 (±1.07)	0.4729
Tree age (year)	75.97 (±3.56)	69.57 (±5.03)	61.40 (±5.67)	0.1145
DBH (cm)	13.21 (±0.52)	14.70 (±0.71)	16.13 (±0.52)	0.0047
Tree height (m)	7.47 (±0.25)	8.99 (±0.33)	9.98 (±0.27)	<0.001

Table 3-2. Macrolichen thalli count with relative abundance (%) in brackets and the transect type lichen species are predominately association with. Significant lichen associations (α = 0.05) are indicated with an asterisk.

Lichen Species	Swamp	Ecotone	Upland	Associated Transect	Indicator Value
Alectoria sarmentosa	743 (61)	248 (25)	135 (14)	Swamp	61.2*
Bryoria spp.	876 (43)	596 (32)	432 (25)	Swamp	42.8*
Cladonia spp.	120 (30)	123 (34)	115 (36)	Ecotone	31.7
Coccocarpia palmicola	329 (44)	202 (50)	20 (6)	Ecotone	27.1
Erioderma pedicellatum	25 (78)	3 (22)	0	Swamp	15.6
Hypogymnia incurvoides	282 (38)	207 (30)	240 (32)	Swamp	38.1
H. physodes	637 (33)	707 (35)	676 (32)	Ecotone	35.0
H. tubulosa	20 (28)	28 (56)	14 (16)	Ecotone	26.4
H. vittata	135 (44)	115 (39)	26 (17)	Swamp	38.5
Imshaugia aleurites	1 (33)	0	2 (67)	Upland	4.4
Lobaria pulmomaria	14 (100)	0	0	Swamp	6.7
L. scrobiculata	1 (20)	4 (80)	0	Ecotone	10.7
Menegazzia subsimilis	0	5 (100)	0	Ecotone	13.3
M. terebrata	3 (14)	16 (76)	2 (10)	Ecotone	20.3
Parmelia squarrosa	1046 (39)	946 (34)	737 (27)	Swamp	38.9
Parmeliella parvula	3 (100)	0	0	Swamp	6.7
Platismatia glauca	1320 (39)	1078 (32)	979 (29)	Swamp	39.3
P. norvegica	425 (33)	356 (33)	388 (34)	Swamp	33.2
Ramalina spp.	4 (32)	10 (20)	15 (48)	Upland	6.4
Sphaerophorus globosus	676 (36)	832 (47)	269 (17)	Ecotone	34.6
Tuckermanopsis orbata	2 (100)	0	0	Swamp	6.7
Usnea spp.	145 (24)	236 (33)	309 (43)	Upland	40.3

Macrolichen richness (Figure 3-2) and diversity (Figure 3-3) were highest in forested swamps, then in the ecotones, and lowest in the upland forest. The Kruskal-Wallis tests revealed significant differences among transect types for richness (*p* = 0.004) but not diversity (*p* = 0.062). Since richness had a significant difference overall, pairwise comparisons were applied to show the differences among transect types and showed significant differences between upland forests and both ecotones (*p* = 0.019) and swamps (*p* = 0.001).

The NMDS analysis and ordination showed separation between the forested swamp and upland forest lichen communities and overlap with the ecotone community (Figure 3-4). A final stress of 0.123 for a three-dimensional solution was concluded after 100 iterations using a random starting seed. Environmental variables were fitted as vectors to the ordination plot with sites lower on axis 2 having the taller, bigger trees and the sites higher on axis 1 were the oldest with more canopy openness (Table 3-3). The results of the perMANOVA showed that the difference in lichen species composition among transect types was significant (*p* = 0.018, Table 3-4).

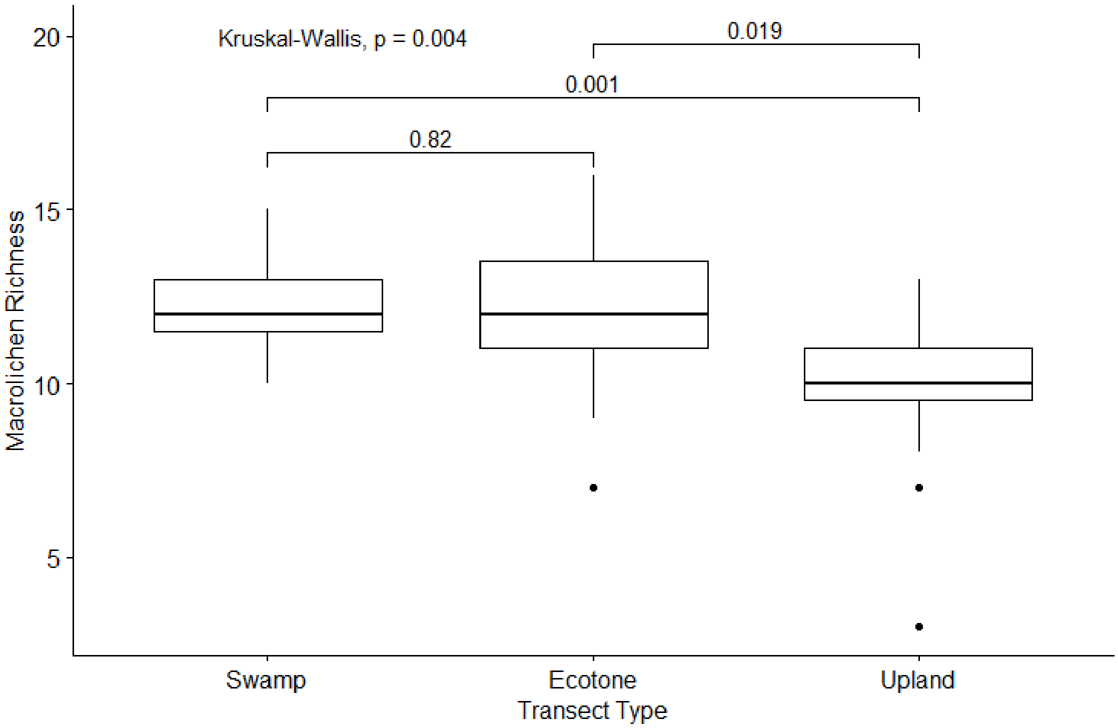

Figure 3-2. Macrolichen richness boxplots displayed by transect type. A Kruskal-Wallis test compared the difference among the three transect types, and pairwise comparisons were used to detect differences between each transect type. Significance levels were set to $\alpha = 0.05$. Boxes represent 25th and 75th quartiles, and whiskers represent the minimum and maximum data points, exclusive of outliers.

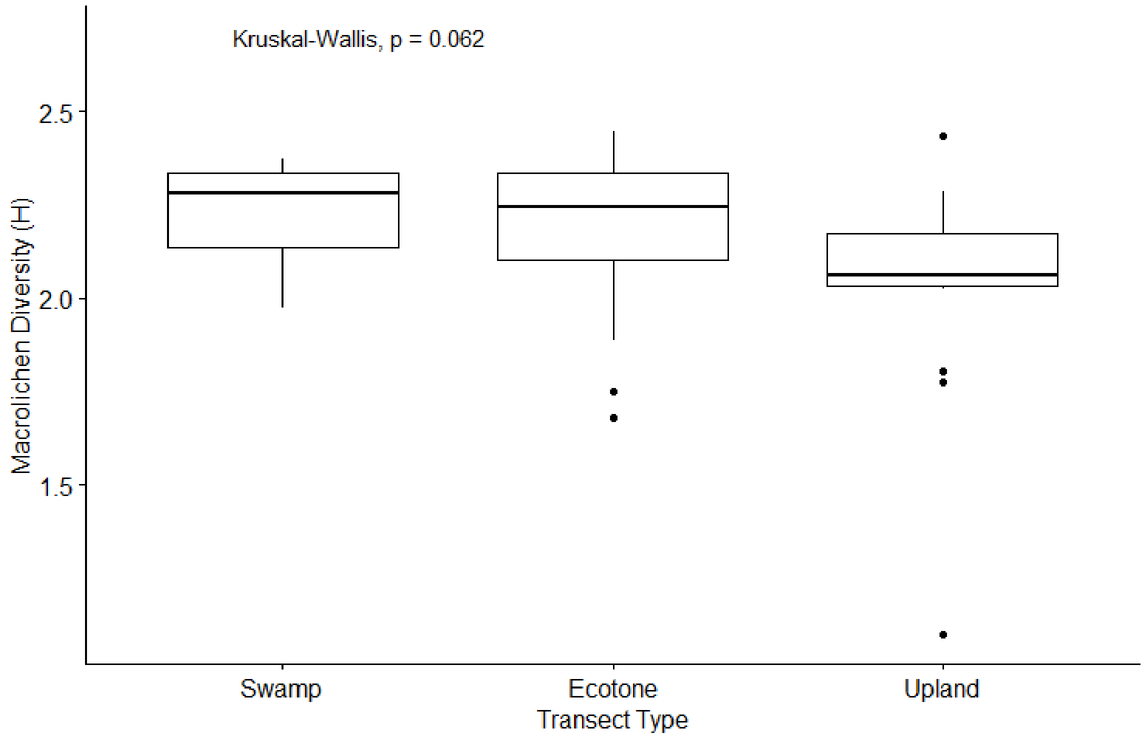

Figure 3-3. Macrolichen Shannon diversity index boxplots displayed by transect type. A Kruskal-Wallis test compared the difference among the three transect types. Significance levels were set to α = 0.05. Boxes represent 25th and 75th quartiles, and whiskers represent the minimum and maximum data points, exclusive of outliers.

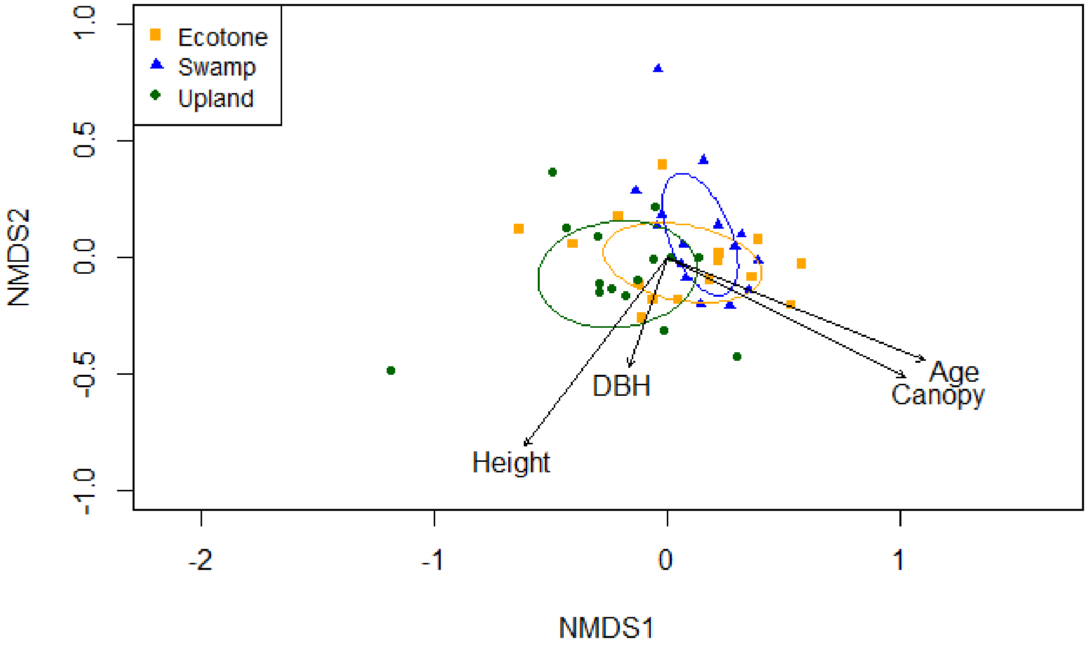

Figure 3-4. Ordination results of the non-metric multidimensional scaling (NMDS) analysis of the macrolichen community composition with 95% confidence interval ellipses around each transect type. A final stress of 0.123 with a three-dimensional solution was concluded. Environmental site variables were fitted as vectors with the vector arrows position indicating the direction of the gradient and length indicating the strength of the gradient.

Table 3-3. Explanatory variable vectors fitted to the non-metric multidimensional (NMDS) scaling analysis plot. The number of permutations was 999 and the significance level was α = 0.05. The NMDS 1 and 2 columns give the cosines of the vectors, and the R^2 values give the squared correlation coefficient. DBH = diameter at breast height.

Variable	NMDS 1	NMDS 2	R^2	*P*
Canopy cover	0.89166	-0.45271	0.3012	0.002
Tree age	0.92745	-0.37396	0.3261	0.001
Tree DBH	-0.32869	-0.94444	0.0567	0.267
Tree height	-0.60186	-0.79860	0.2356	0.008

Table 3-4. Results of the non-parametric multivariate analysis of variance (perMANOVA) using Bray-Curtis dissimilarity for macrolichen community composition by transect type (swamp, ecotone, upland). Significance level was α = 0.05).

Source	Df	SS	MS	F	R^2	*P* (>F)
Transect	2	0.21351	0.106755	2.1088	0.0913	0.018
Residuals	42	2.12618	0.050623	-	0.9087	-
Total	44	2.33969	-	-	1.0000	-

3.5 Discussion

Forested swamps had higher macrolichen richness and diversity than adjacent upland forests. Forest continuity plays an important role in lichen communities (McMullin and Wiersma, 2019) and forests with less disturbance, and therefore more continuity, have higher epiphytic lichen diversity (Fritz et al., 2008). However, the tree age among transect types in this study was not significantly different. Fire is likely less frequent in forested swamps compared to upland forests; however, fire is not a dominant disturbance in Newfoundland's boreal forests (Arsenault et al., 2016b). Trees were shorter and smaller in the swamps and taller and bigger in the upland forests despite similar canopy cover and having trees of similar age. This phenomenon is posited to happen because of stunted growth of the trees in the wetland, in this case swamps, and is common in northern forested wetlands (Dahl and Zoltai, 1997).

Contrary to my hypothesis, ecotone transects did not have higher macrolichen richness and diversity as has been observed in studies with other taxa (Kark and van Rensburg, 2006). This is likely because the transition between the forested swamp and the upland forest was small and therefore could not support additional species. The bigger difference was between the swamp and ecotone macrolichen communities and the upland forest macrolichen communities. An ecotone between two more extreme environments, (e.g., open bog-forest edge) might be expected to have elevated epiphytic lichen richness and diversity, but there is also the possibility of a negative edge influence depending on what kind of edge (Esseen, 2006) or no significant influence (Harper et al., 2015). Also, in boreal wetland forested areas, the forested swamp itself could be considered an ecotone habitat between the open wetland and the upland forest (Kuusinen, 1996).

Macrolichen communities in forested swamps, ecotones, and upland forests were significantly different. The ordination plot of the NMDS scores and the significant perMANOVA results show the distinct groups of macrolichen composition that could be from the relative humidity difference between the habitats. Beetle species community composition in habitats with different humidity levels have shown distinct separation of groups in ordination plots (Dufrêne and Legendre, 1997), and even within different wetland habitats, plants form distinct groups based on different water and humidity levels (Flinn et al., 2008). The similarity of forested swamp and upland forest macrolichen communities with the ecotone community reflects the fact that the majority of lichen species that were found in these habitats were the same as those found in the swamp and upland habitats.

A limitation of this study was looking only at macrolichens and not including microlichens in the surveys. Microlichens respond to different aspects of ecosystems and may have demonstrated more of a difference in the ecotone where I did not detect an increase in richness or diversity. Restricting the macrolichen survey area from 0.9 to 1.9 m on the tree bole may have excluded some of the transitional lichens found between these habitat types. Also, identifying the fruticose lichens *Alectoria*, *Usnea*, and *Bryoria* to genus only could not have picked up on subtle differences leaving diversity patterns undetected. Future research should include applying this design to different regions and forest types as well as using lichens as part of biodiversity surveys.

The results of the study support the hypothesis of higher macrolichen richness and diversity in the forested swamp but did not support the alternative hypothesis of higher richness and diversity in the ecotone. The results also support the hypothesis that macrolichen community composition of forested swamps and upland forests is distinct and overlaps with ecotones. This research contributes to understanding fine-scale patterns of lichens distribution in heterogeneous habitats such as wetland-forest mosaic landscapes.

The knowledge gained from this research that there is higher macrolichen richness and diversity in forested swamps compared to non-wetland upland forests highlights the importance of these wetlands. They contribute to higher landscape biodiversity and act as refuges for rare lichen species such as the boreal felt lichen (*Erioderma pedicellatum*). Because of these contributions, forested wetlands are important areas of conservation in a forested landscape and should be treated differently than their adjacent upland forests.

3.6 References

Anderson, M. J. (2001). A new method for non-parametric multivariate analysis of variance. Austral Ecology 26: 32–46.

Arsenault, A., Leblanc, R., Earle, E., Brooks, D., Clarke, B., Lavigne, D., and Royer, L. (2016). Unravelling the past to manage Newfoundland's forests for the future 92: 487–502.

Berglund, H. (2004). Biodiversity in fragmented boreal forests - assessing the past, the present and the future. Department of Ecology and Environmental Science, Umea University, Sweden.

Brodo, I. M. (2016). Keys to Lichens of North America. New Haven and London: Yale University Press.

Brodo, I. M., Sharnoff, S. D., and Sharnoff, S. (2001). Lichens of North America. New Haven and London: Yale University Press.

Dahl, T. E. and Zoltai, S. C. (1997). Forested northern wetlands of North America. In Northern forested wetlands: ecology and management (Vol. 5667, pp. 3–18).

Damman, A. W. H. (1983). An ecological subdivision of the island of Newfoundland. In G. R. South (Ed.), Biogeography and Ecology of the Island of Newfoundland (pp. 163–206). The Hague, the Netherlands: Dr. W Junk Publishers.

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

Ellis, C. J. (2012). Lichen epiphyte diversity: A species, community and trait-based review. Perspectives in Plant Ecology, Evolution and Systematics 14: 131–152.

Esseen, P.-A. (2006). Edge influence on the old-growth forest indicator lichen Alectoria sarmentosa in natural ecotones. Journal of Vegetation Science 17: 185–194.

Flinn, K. M., Lechowicz, M. J., and Waterway, M. J. (2008). Plant species diversity and composition of wetlands within an upland forest. American Journal of Botany 95: 1216–1224.

Fritz, Ö., Gustafsson, L., and Larsson, K. (2008). Does forest continuity matter in conservation? A study of epiphytic lichens and bryophytes in beech forests of southern Sweden. Biological Conservation 141: 655–668.

Grytnes, J. A., Heegaard, E., and Ihlen, P. G. (2006). Species richness of vascular plants, bryophytes, and lichens along an altitudinal gradient in western Norway. Acta Oecologica 29: 241–246.

Harper, K. A., Macdonald, S. E., Mayerhofer, M. S., Biswas, S. R., Esseen, P. A., Hylander, K., … Bergeron, Y. (2015). Edge influence on vegetation at natural and anthropogenic edges of boreal forests in Canada and Fennoscandia. Journal of Ecology 103: 550–562.

Hauck, M., Javkhlan, S., Lkhagvadorj, D., Bayartogtokh, B., Dulamsuren, C., and Leuschner, C. (2012). Edge and land-use effects on epiphytic lichen diversity in the forest-steppe ecotone of the Mongolian Altai. Flora: Morphology, Distribution, Functional Ecology of Plants 207: 450–458.

Hinds, J. W., and Hinds, P. L. (2007). Macrolichens of New England. Bronx, NY: New York Botanical Garden Press.

Hornberg, G., Zackrisson, O., Segerstrom, U., Svensson, B. W., Ohlson, M., and Bradshaw, R. H. W. (1998). Boreal swamp forests. BioScience 48: 795–802.

Kark, S. (2013). Effects of ecotones on biodiversity. In Encyclopedia of Biodiversity: Second Edition (pp. 142–148). Reference module for life sciences: Elsevier.

Kark, S. and van Rensburg, B. J. (2006). Ecotones: Marginal or central areas of transition? Israel Journal of Ecology & Evolution 52: 29–53.

Kraichak, E., Pope, R., and Wheelwright, N. T. (2009). Habitat associations of macrolichens on a boreal island in the Bay of Fundy, New Brunswick, Canada. The Bryologist 112: 762–772.

Kuusinen, M. (1996). Importance of spruce swamp-forests for epiphyte diversity and flora on *Picea abies* in southern and middle boreal Finland. Ecography 19: 41–51.

McCune, B. and Grace, J. B. (2002). Analysis of Ecological Communities (3rd ed.). Gleneden Beach, OR: MjM Software Design.

McMullin, R. T., Ure, D., Smith, M., Clapp, H., and Wiersma, Y. F. (2017). Ten years of monitoring air quality and ecological integrity using field-identifiable lichens at Kejimkujik National Park and National Historic Site in Nova Scotia, Canada. Ecological Indicators 81: 214–221.

Nascimbene, J. and Spitale, D. (2017). Patterns of beta-diversity along elevational gradients inform epiphyte conservation in alpine forests under a climate change scenario. Biological Conservation 216: 26–32.

National Wetlands Working Group (1997). The Canadian wetland classification system. Waterloo, Canada: Wetlands Research Branch, University of Waterloo.

Oksanen, J., Guillaume Blanchet, F., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., … Wagner, H. (2017). Vegan: Community ecology package. R package version 2.4-6.

Peck, J. E. (2016). Multivariate analysis for ecologists: Step-by-step (2nd ed.). Gleneden Beach, OR: MjM Software Design.

Purvis, W. (2000). Lichens. The Natural History Museum, London: Smithsonian Institution Press in association with the Natural History Museum, London.

R Core Team (2018). R: A language and environment for statistical computing. Vienna Austria: R Foundation for Statistical Computing.

Roberts, D. W. (2016). labdsv: Ordination and multivariate analysis for ecology. R package version 1.8-0.

Sjöberg, K. and Ericson, L. (1997). Mosaic boreal landscapes with open and forested wetlands. Ecological Bulletins 46: 48–60.

Townsend, P. A. (2001). Mapping seasonal flooding in forested wetlands using multi-temporal Radarsat SAR. Photogrammetric Engineering and Remote Sensing 67: 857–864.

Vaisanen, R. (1992). Distribution and abundance of diurnal Lepidoptera on a raised bog in southern Finland. Annales Zoologici Fennici 29: 75–92.

Chapter 4 Summary

4.1 Summary of Results

In this study, I started to address the lack of and need for research on lichens in forested wetlands. This study was the first on the island of Newfoundland to specifically look at what macrolichens live in forested wetlands and if there was a difference among lichen communities between habitats, ecoregions, and non-wetland forests. The importance of this research is two-fold, understanding lichen community composition patterns and forested wetland ecology. Spatial patterns of lichen communities and how they are distributed between wetland habitats, non-wetland habitats, and across regions is important for future monitoring and identifying areas of high biodiversity. By using lichens as study species, we can start seeing some of the differences in community structure between forested wetland classes and surrounding non-wetland habitats. This research starts to answer the question of whether we can use lichens to study forested wetlands and where the next steps need to happen to make lichens a tool to study forested wetland ecosystems.

In chapter 2, I compared macrolichen communities among different forested wetland classes (forested bog, fen, swamp) and across three ecoregions. The three forested wetland classes had varying levels of macrolichen diversity with forested fens having the highest macrolichen diversity. The three ecoregions also had varying levels of macrolichen diversity with the Northern Peninsula Forest having the highest macrolichen diversity. However, I found there to be no significant difference in macrolichen diversity among forested wetland classes or across ecoregions. The geographic scale, as opposed to the habitat scale, is more of an influence on forested wetland macrolichen communities. However, this does not mean the most distantly separated lichen communities will differ more, as seen with the coastal regions, the Avalon Forest and the Northern Peninsula Forest, having more similar lichen communities than the inland Central Newfoundland communities.

I also identified macrolichen indicator species for forested wetland classes and ecoregions. Forested bogs had *Alectoria sarmentosa* and forested fens had *Usnea longissima* as macrolichen indicator species. Regarding macrolichen indicators for each of the ecoregions, for the Avalon Forest *Bryoria* spp., *Cladonia* spp., *Sphaerophorus globosus*, and *Usnea longissima* were indicator species. For the Central Newfoundland Ecoregion, *Hypogymnia vittata* was an indicator species and for the Northern Peninsula, *H. incurvoides* and *Vulpicida pinastri* were indicator species. However, all the potential indicator species were also observed in the surrounding forests potentially decreasing their validity for forested wetland indicator but also highlighting the need for more research.

In chapter 3, I compared forested swamp macrolichen communities to non-wetland upland forests and ecotones of the swamp-upland margin. I found differences between the habitats in macrolichen richness and diversity. Overall, macrolichen richness was significantly different among the three transect types with forested swamps having the highest macrolichen richness, followed by ecotones, and lastly upland forests. For macrolichen diversity, overall there was not a significant difference among transect types but forested swamps still had the highest, followed by ecotones, and lastly upland forests. I did not see elevated richness or diversity levels in the ecotone as alternatively hypothesized. I also observed some interesting patterns when looking at the macrolichen community data in a non-metric multidimensional scaling (NMDS) analysis ordination plot. Forested swamp and upland forest transects formed separate distinct groups and both groups overlapped with ecotone transects, and all three groups of macrolichen community composition were significantly different from each other.

4.2 Limitations

Limitations to this research varied with each chapter. In the second chapter, not conducting lichen surveys in non-wetland areas to further verify the indicator species was a limitation. I collected macrolichen data within different forested wetland classes but not in other forest types present on the landscape. This study design meant that when I conducted the Indicator Species Analysis, the results could only tell me if the forested wetland classes have indicator species and not if these indicators were exclusive to the forested wetlands and not found in the non-wetland forests. De Cáceres et al. (2010) highlight that Indicator Species Analysis should incorporate the different niche breadths of species, because if the analysis is undertaken on individual groups of sites the analysis may fail to reveal species that are connected to the prevalent conditions in two or more groups of sites. Improving the analysis should include considering all possible groups of sites, and in the case of this study should include different wetland and non-wetland forest sites. However, time and logistics of fieldwork, as well as funding being constrained to work on forested wetlands, did not allow for surveys in adjacent upland forests across the island.

Regarding both chapters of my thesis, not identifying the hair lichens to species could have reduced my ability to detect differences, if they were present. Lichens in the genera *Usnea* and *Bryoria* were detected as indicator species of a specific wetland habitat or ecoregion. If I had identified each to the species level, I could have determined which species were the indicators or if all the species in that genus were indicators. The reason why I did not identify *Usnea* and *Bryoria* genera to species level was that in the boreal forests of Newfoundland they are abundant and difficult to identify to species. Usually chemical tests are required to discriminate species, and this is not practical in the field. I decided to group the species within these two genera to increase ease of identification and decrease species identification error. In doing so, I may have reduced statistical differentiation of macrolichen communities. Kraichak et al. (2009) studied macrolichen habitat associations in the boreal forest and found *Bryoria fuscescens* to be an indicator of mixed coniferous forests but other *Bryoria* species, *B. nadvornikiana* and *B. trichodes*, were not.

Another limitation to both chapters is only surveying lichens between 0.9-1.9 m on the tree bole instead of surveying below and above (branches included). I chose to use the survey area of 0.9-1.9 m on the tree bole because of optimizing the detection of arboreal lichens without the use of a ladder or climbing equipment. Surveying for lichen under 0.9 m would likely detect terricolous lichens and surveying above 1.9 m would need specialized equipment. Lichen richness and diversity has been shown to vary with the vertical gradient of the trees with increasing levels with increasing height from the ground (Marmor et al., 2013). Also, older tree canopy lichen communities can often have distinct communities with rare lichens (McCune et al., 2000). However, the need for repeatable straightforward lichen surveying is important for management, which is why the survey area of 0.9-1.9 m on the tree bole was appropriate for this study.

4.3 Conservation Implications and Future Research

The conservation implications of this study are two-fold. There are implications to the field of forested wetland research and the field of lichen research. Both fields presented knowledge gaps that my research will help address with room for future research to add to the increasing knowledge and understanding of macrolichen and forested wetland ecology.

My results suggest that lichens reflect large- and small-scale differences within and between forested wetlands on the landscape. Even though different forested wetland habitats did not show significantly different macrolichen communities, when comparing forested swamp macrolichen communities to the adjacent upland forest macrolichen communities, I was able to show that the forested swamps had higher macrolichen richness and diversity. This is important for forested wetland conservation because it highlights that some forested wetlands have higher biodiversity than the surrounding non-wetland forests. This should translate into more effort to conserve forested wetlands as they could be areas of high biodiversity for other organisms as well as lichens, and potentially act as habitat refugia. Wetlands within forested landscapes have been shown to provide disproportionately high levels of plant diversity which make important contributions to landscape-level diversity (Flinn et al., 2008). Understanding the community of organisms that rely on forested wetlands is an important area of future research.

Future research on lichens in forested wetlands should include additional studies along with long-term monitoring. My research provides the first baseline lichen data for forested wetlands on the island of Newfoundland, Canada, and can be used as a comparison in future lichen surveys. Lichens are candidates for studying long-term forest integrity (McCune, 2000; McMullin and Wiersma, 2019) and can be used to study changes in climate (Nascimbene and Spitale, 2017) and air quality (McMullin et al., 2017; Seed et al., 2013; Will-Wolf et al., 2015). The lichen communities in my study can be used to monitor changes in forested wetlands as well as to compare to other forested wetlands and non-wetland forests in different boreal forests. The island of Newfoundland is a part of the boreal forest biome which is Canada's largest vegetation zone making up 55% of the country's landmass (Brandt et al., 2013). Research conducted in boreal forest wetlands of Newfoundland could be broadly applied to other boreal forested wetland ecosystems.

Forested wetlands are important and valuable ecosystems that need more research and understanding. Macrolichens are a large component of forested wetlands and have been shown to be good candidates to study forested wetland dynamics and ecology. With more research on macrolichens in forested wetlands, a better understanding of wetland dynamics along with practical surveying tools, such as indicator species, is possible. This research could then provide a standardized technique to monitoring forested wetlands using lichens on a global scale. With an increasingly unpredictable climate, the reliance on wetland ecosystem services also increases (Mitsch and Gossilink, 2000) highlighting the importance of research within all wetland ecosystems.

4.4 References

Brandt, J. P., Flannigan, M. D., Maynard, D. G., and Thompson, I. D. (2013). An introduction to Canada’s boreal zone: Ecosystem processes, health, sustainability, and environmental issues. Environmental Reviews 21: 207–226.

De Cáceres, M., Legendre, P., and Moretti, M. (2010). Improving indicator species analysis by combining groups of sites. Oikos 119: 1674–1684.

Flinn, K. M., Lechowicz, M. J., and Waterway, M. J. (2008). Plant species diversity and composition of wetlands within an upland forest. American Journal of Botany 95: 1216–1224.

Kraichak, E., Pope, R., and Wheelwright, N. T. (2009). Habitat associations of macrolichens on a boreal island in the Bay of Fundy, New Brunswick, Canada. The Bryologist 112: 762–772.

Marmor, L., Tõrra, T., Saag, L., Leppik, E., and Randlane, T. (2013). Lichens on *Picea abies* and *Pinus sylvestris* - From tree bottom to the top. The Lichenologist 45: 51–63.

McCune, B. (2000). Lichen communities as indicators of forest health. The Bryologist 103: 353–356.

McCune, B., Rosentreter, R., Ponzetti, J. M., and Shaw, D. C. (2000). Epiphytic habitats in an old conifer forest in western Washington, USA. The Bryologist 103: 417–427.

McMullin, R. T., Ure, D., Smith, M., Clapp, H., and Wiersma, Y. F. (2017). Ten years of monitoring air quality and ecological integrity using field-identifiable lichens at Kejimkujik National Park and National Historic Site in Nova Scotia, Canada. Ecological Indicators 81: 214–221.

McMullin, R. T. and Wiersma, Y. F. (2019). Out with OLD growth, in with ecological contiNEWity: new perspectives on forest conservation. Frontiers in Ecology and the Environment 17: 176-181.

Mitsch, W. J. and Gossilink, J. G. (2000). The value of wetlands: Importance of scale and landscape setting. Ecological Economics 35: 25–33.

Nascimbene, J. and Spitale, D. (2017). Patterns of beta-diversity along elevational gradients inform epiphyte conservation in alpine forests under a climate change scenario. Biological Conservation 216: 26–32.

Seed, L., Wolseley, P., Gosling, L., Davies, L., and Power, S. A. (2013). Modelling relationships between lichen bioindicators, air quality and climate on a national scale: Results from the UK OPAL air survey. Environmental Pollution 182: 437–447.

Will-Wolf, S., Jovan, S., Neitlich, P., Peck, J. E., and Rosentreter, R. (2015). Lichen-based indices to quantify responses to climate and air pollution across northeastern USA. The Bryologist 118: 59–82.

Appendices

Appendix 1. Supplemental map showing the spatial distribution of chapter 2 sample sites.

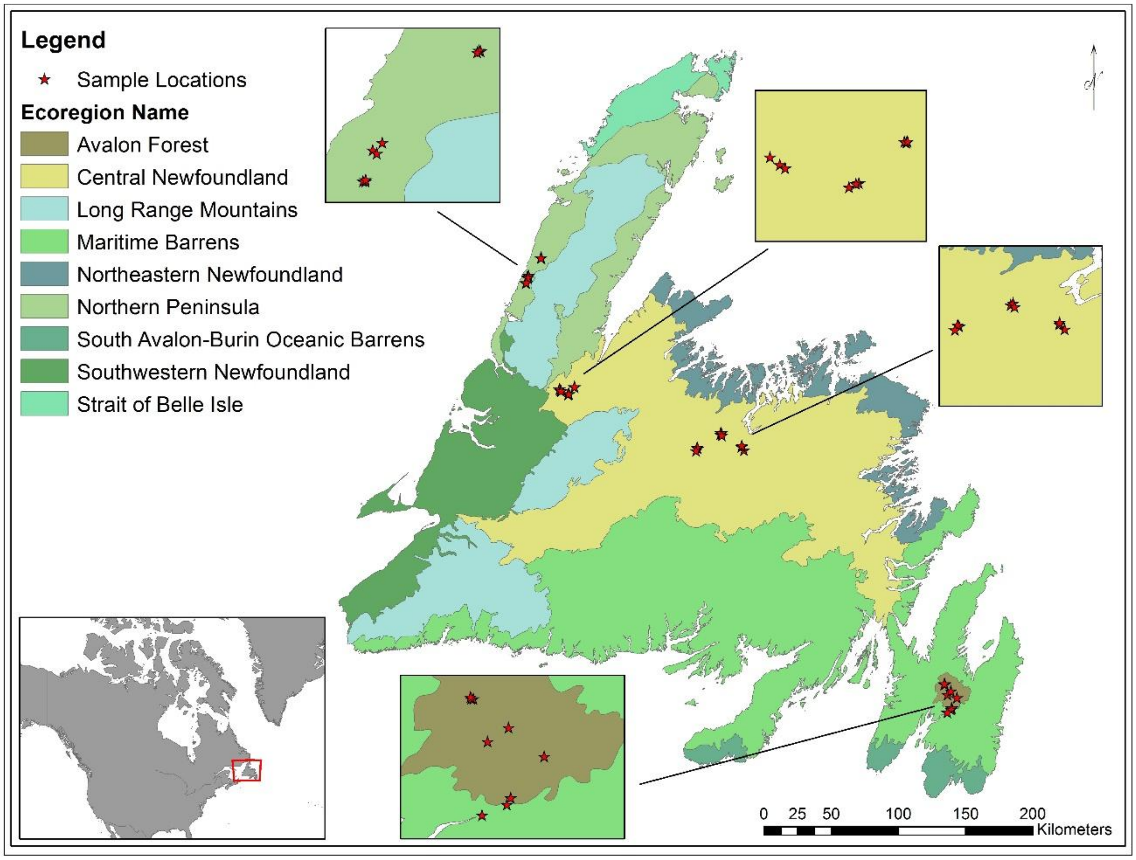

Figure 1-A1. Map of the forested wetland (bog, fen, swamp) sites showing the ecoregions of Newfoundland. Each inset is a closeup of the sites. There were nine forested wetland sites in the Northern Peninsula, eighteen sites in the Central Newfoundland, and nine sites in the Avalon Forest Ecoregion

Appendix 2. Supplement information on geographic locations of chapter 2 sample sites. Abbreviations for study sites are as follows: AVL = Avalon, GFW = Grand Falls-Windsor, DLK = Deer Lake, GMN = Gros Morne. Abbreviations for ecoregions are as follows: AF = Avalon Forest, CN = Central Newfoundland, NP = Northern Peninsula.

Plot Name	Study Site	Ecoregion	Forested Wetland Class	Latitude	Longitude
AVL-FB-1	AVL	AF	Bog	47.32282	-53.3518
AVL-FF-1	AVL	AF	Fen	47.30018	-53.3857
AVL-FS-1	AVL	AF	Swamp	47.36967	-53.409
AVL-FB-2	AVL	AF	Bog	47.36951	-53.4123
AVL-FF-2	AVL	AF	Fen	47.37231	-53.4134
AVL-FS-2	AVL	AF	Swamp	47.18105	-53.3947
AVL-FB-3	AVL	AF	Bog	47.19821	-53.3547
AVL-FF-3	AVL	AF	Fen	47.20896	-53.3485
AVL-FS-3	AVL	AF	Swamp	47.27615	-53.2943
GFW-FB-1	GFW	CN	Bog	48.98498	-55.8441
GFW-FF-1	GFW	CN	Fen	48.97858	-55.8376
GFW-FS-1	GFW	CN	Swamp	49.07357	-55.6083
GFW-FB-2	GFW	CN	Bog	48.96531	-55.3684
GFW-FF-2	GFW	CN	Fen	48.99598	-55.3906
GFW-FS-2	GFW	CN	Swamp	48.98812	-55.3916
GFW-FB-3	GFW	CN	Bog	49.06514	-55.5918
GFW-FF-3	GFW	CN	Fen	49.08122	-55.5979
GFW-FS-3	GFW	CN	Swamp	48.96279	-55.8563
DLK-FB-1	DLK	CN	Bog	49.36723	-57.2539
DLK-FF-1	DLK	CN	Fen	49.33702	-57.1566
DLK-FS-1	DLK	CN	Swamp	49.35868	-57.2426
DLK-FB-2	DLK	CN	Bog	49.3378	-57.1534
DLK-FF-2	DLK	CN	Fen	49.35461	-57.2367
DLK-FS-2	DLK	CN	Swamp	49.33332	-57.1645
DLK-FB-3	DLK	CN	Bog	49.38472	-57.1016
DLK-FF-3	DLK	CN	Fen	49.38379	-57.0995
DLK-FS-3	DLK	CN	Swamp	49.38511	-57.0981
GMN-FB-1	GMN	NP	Bog	50.11507	-57.6072
GMN-FF-1	GMN	NP	Fen	50.12466	-57.5945
GMN-FS-1	GMN	NP	Swamp	50.1104	-57.6019
GMN-FB-2	GMN	NP	Bog	50.07406	-57.6198
GMN-FF-2	GMN	NP	Fen	50.07359	-57.6169
GMN-FS-2	GMN	NP	Swamp	50.07587	-57.6163
GMN-FB-3	GMN	NP	Bog	50.24612	-57.467

GMN-FF-3	GMN	NP	Fen	50.24522	-57.4651
GMN-FS-3	GMN	NP	Swamp	50.24355	-57.4695

Appendix 3. Representative site photos of forested bogs, fens, and swamps and their ground and shrub cover from chapter 2.

Figure 1-A3. The forested bog sites were in the periphery forests of open bogs.

Figure 2-A3. Ground and shrub cover of the forested bog sites.

Figure 3-A3. Representative photo of the forested fen sites.

Figure 4-A3. Ground and shrub cover of the forested fen sites.

Figure 5-A3. Representative photo of the forested swamp sites.

Figure 6-A3. Ground and shrub cover of the forested swamp sites.

Appendix 4. Supplement information on geographic locations of chapter 3 sample sites.

Site Names	Transect	Latitude	Longitude
S1-ET	Ecotone	47.3695	-53.4088
S1-FS	Swamp	47.36965	-53.4089
S1-UF	Upland	47.36924	-53.4084
S2-ET	Ecotone	47.27613	-53.2942
S2-FS	Swamp	47.27619	-53.2943
S2-UF	Upland	47.27576	-53.2942
S3-ET	Ecotone	47.36811	-53.4446
S3-FS	Swamp	47.36779	-53.4448
S3-UF	Upland	47.3683	-53.4446
S4-ET	Ecotone	47.27504	-53.249
S4-FS	Swamp	47.27515	-53.2487
S4-UF	Upland	47.27474	-53.2493
S5-ET	Ecotone	47.25723	-53.2837
S5-FS	Swamp	47.2569	-53.2837
S5-UF	Upland	47.25755	-53.2837
S6-ET	Ecotone	47.29191	-53.3459
S6-FS	Swamp	47.29189	-53.346
S6-UF	Upland	47.29171	-53.3457
S7-ET	Ecotone	47.18128	-53.3943
S7-FS	Swamp	47.1813	-53.3946
S7-UF	Upland	47.18113	-53.3939
S8-ET	Ecotone	47.23315	-53.3584
S8-FS	Swamp	47.23314	-53.3579
S8-UF	Upland	47.2333	-53.3585
S9-ET	Ecotone	47.22229	-53.3164
S9-FS	Swamp	47.22213	-53.3162
S9-UF	Upland	47.22252	-53.3162
S10-ET	Ecotone	47.40487	-53.419
S10-FS	Swamp	47.40522	-53.4193
S10-UF	Upland	47.40469	-53.4189
S11-ET	Ecotone	47.3367	-53.4137
S11-FS	Swamp	47.33707	-53.4142
S11-UF	Upland	47.33643	-53.4131
S12-ET	Ecotone	47.26602	-53.5005
S12-FS	Swamp	47.26578	-53.4996
S12-UF	Upland	47.26582	-53.5008
S13-ET	Ecotone	47.36154	-53.2667
S13-FS	Swamp	47.36137	-53.2665
S13-UF	Upland	47.3619	-53.267
S14-ET	Ecotone	47.32072	-53.2333
S14-FS	Swamp	47.32066	-53.2334

S14-UF	Upland	47.32072	-53.2329
S15-ET	Ecotone	47.26967	-53.3335
S15-FS	Swamp	47.26945	-53.3351
S15-UF	Upland	47.26948	-53.3336

Appendix 5. Representative site photos of the swamp, ecotone, and upland forest transect areas from chapter 3.

Figure 1-A5. Representative photo of the forested swamp transect area.

Figure 2-A5. Representative photo of the ecotone transect area.

Figure 3-A5. Representative photo of the upland forest transect area.