Gastropod Grazing of Epiphytic Lichens in Western Newfoundland

By © Katherine Flores

A Thesis submitted to the School of Graduate Studies in partial fulfillment of the

requirements for the degree of

Master of Science (Environmental Science)

Memorial University of Newfoundland

June 2021

St. John's, Newfoundland and Labrador

Abstract

Overgrazing by introduced gastropods has been identified as a potential threat to some rare or threatened epiphytic foliose lichens; however, gastropod grazing severity has not been evaluated within Newfoundland. *Lobaria pulmonaria* is locally abundant in Newfoundland but considered rare and threatened in parts of the U.S.A and Europe. To evaluate gastropod grazing severity and describe the tree-climbing gastropod community we conducted lichen diversity and lichenivory surveys and used two methods of gastropod sampling. To determine how lichen species diversity affects lichen grazing of *L. pulmonaria*, we conducted cafeteria feeding experiments using native and introduced snail species and common lichen species from our study area. We found that within the study area, some lichens including *L. pulmonaria* were severely overgrazed, and that tree-climbing gastropod abundance increased with greater grazing severity. We found that lichenivory severity was driven by tree-level factors and identified the most common tree-climbing slugs within the study area. Feeding experiment results showed that snails consumed greater lichen biomass with greater species diversity; however, distinct preferences for specific lichen species were apparent. Grazing severity of *L. pulmonaria* decreased with greater lichen species diversity, which suggests that *L. pulmonaria* may be more resistant to gastropod grazing in forests with higher foliose lichen species diversity.

General Summary

Grazing by introduced snail and slug species may be of concern for rare or threatened lichens. It is important to identify the amount of lichen grazing occurring today, to monitor lichen grazing in the future. After completing field surveys, we found that some lichens in our study area were extensively grazed by slugs and snails. Using traps and searches, we identified five species of tree-climbing slug and snail species that are probably lichenivores. The amount of grazing on lichens depended on the type of host tree, and the location of the lichen on the tree. We completed lab experiments to test whether the number of lichen species on trees affected lichen grazing by snails and found that in the presence of a larger number of lichen species, some lichens were consumed less. Therefore, some lichens are more protected from snail grazing when they occur within a community of other lichen species.

Acknowledgments

Table of Contents

List of Tables

List of Figures

1 Introduction and Overview

Lichens are the result of a symbiosis, an intimate bond of a fungal species and a green alga (chlorolichen), a cyanobacterium (cyanolichen) or both green algae and cyanobacteria (cephalolichen) (Nash 2008). Lichens are a source of great biodiversity and are increasingly being considered a complex ecosystem in themselves (Hawksworth & Grube 2020). They also provide a food source for many animal species (Henderson & Hackett 1986), and habitat for a plethora of endophytic fungi (U'Ren *et al.* 2010), basidiomycetous yeasts (Spribille *et al.* 2016; Černajová & Škaloud 2019; Hawksworth & Grube 2020), bacteria, and invertebrates (Hodkinson & Lutzoni 2009).

Lichens can be categorized by their growth form and size (Nash 2008); in this study I focus on foliose macrolichens. Foliose refers to the leafy nature of some lichen thalli with an upper and lower surface and often distinct attachment organs (i.e., rhizines), while macro refers to the large size of the thallus compared to microlichens (crustose lichens). Crustose lichens are attached to a substrate by the entire lower surface or sometimes grow within the substrate. Many lichens grow epiphytically on the surface of trees, but they do not derive their nutrition directly from their host other than some enrichment from stemflow and canopy throughfall (Goward and Arsenault 2000). Instead, epiphytic lichens rely on what they can obtain from the sun through the act of photosynthesis, and from the air and rain from enrichment (Johansson 2008).

Most lichens are slow growing and sessile (cf. vagrant lichen) and are often adapted to specific environments, any environmental change can therefore have negative consequences for highly specialized lichens species. Because of their sensitivity many epiphytic lichen species are good ecological indicators of environmental change such as air pollution and acid rain (Ellis &

Coppins 2006), or habitat disturbances such as timber harvesting (Ellis & Coppins 2006; Hauck 2009). This study will investigate another threat to foliose tree-lichens, which is gastropod lichenivory.

1.1 Species Descriptions

This study focuses on the foliose tree-lichen, *Lobaria pulmonaria* (L.) Hoffm. Although this species is locally abundant in Newfoundland, *L. pulmonaria* is rare or threatened in many parts of the world. The decline of *L. pulmonaria* in Europe, and to a lesser degree North America, is due to its sensitivities to forest fragmentation and air pollution (Rose 1988; Gauslaa 1995; Richardson & Cameron 2004) and recently also climate change (Nascimbene *et al.* 2016). The level of decline seen in parts of Europe threatens the genetic differentiation of the species at the continental scale (Scheidegger *et al.* 2012) with consequences for future resilience to habitat disturbance and climate change (Scheidegger & Werth 2009).

Lobaria pulmonaria is an indicator of undisturbed forest ecosystems and forest continuity (Rose 1974; Andersson & Appelqvisit 1987; Edman *et al.* 2007; Bolli *et al.* 2008; Liira & Sepp 2009). It is often associated with rare or endangered lichen species (Campbell & Fredeen 2004; Pykälä 2004; Nascimbene *et al.* 2010) and has been identified as an indicator of high ecosystem biodiversity within the forested environments where it is found (Gauslaa 1994).

This species often occurs in large colonies, where multiple lichen thalli occur together in a sprawling formation (**Fig. 1.1**). It has a foliose thallus, 5-15 cm broad with lobes 1-3 cm broad. The upper medullary layer contains the green algal photobiont providing the greenish colour often blended with brown cortical fungal melanin, while blue-green cyanobacteria occur in cephalodia within the thallus. The upper surface features a network of ridges giving it a lung like

appearance with soredia and isidia in roundish spots on the ridges and margins. The lower

surface of the lichen is white-brown and tomentose (McCune & Geiser 2009). The morphology

of *L. pulmonaria* looks like a lung and the lichen is commonly known as the lungwort (**Fig 1.1**).

Lobaria pulmonaria is a cephalolichen formed from an ascomycete fungal species as well as a

cyanobacterial species and a green algal species. *Lobaria pulmonaria* often requires mature and

open forests to grow and thrive; favors rich and wet forested environments such as riparian

habitat, or coastal areas which receive diluted ocean spray (Goward and Arsenault 2000).

Figure 1.1. Photograph of *Lobaria pulmonaria* (*Credit. K. Flores*) in the Blue Gulch Watershed in Pasadena on the island of Newfoundland.

1.2 Blue Gulch Watershed Description

The Blue Gulch Watershed is in Pasadena in Western Newfoundland. The watershed provides a water source for the town of Pasadena and so it is protected from recreational and industrial activities that could pollute the water (Town of Pasadena 2018). The area is included in Corner Brook sub-region of the Western Newfoundland ecoregion, characterized by rugged topography upon a slate and limestone bedrock which produces rich and productive soils (Damman 1983). This sub-region features dense boreal forest dominated by *Abies balsamea* (L.) Mill. The area coincides with the northern limit for the tree species *Pinus strobus* L*., Acer rubrum* L*.,* and *Populus tremuloides* Michx. The climate is cool and wet with an annual average temperature of 4 and 7°C and the annual precipitation between 800 and 1200 mm (Environment Canada 2015).

1.3 Lichenivory

Lichenivory, or herbivory of lichens, was first documented in 1791 by Jacques Brez in a list of forage for arthropods such as mites and moths (Brez 1791). Lichenivory is influenced by various factors including the environment where the lichen is found, the nutritional value of the lichen thallus and any defensive adaptations the lichen may have (Asplund *et al.* 2010). Several different animals are known to graze lichens, including some mammals, insects, mites (Gerson & Seaward 1977; Seyd & Seaward 1984; Asplund & Wardle 2017), and gastropods (Baur *et al.* 1995, 2000). Gastropods are capable of inflicting severe damage to the lichen thallus (Coker 1967; Fröberg *et al.* 1993; Benesperi & Tretiach 2004; Asplund & Gauslaa 2007; Gauslaa 2008), whereas other lichenivores (except mammals) may only inflict superficial surface damage that does not affect the survival of the lichen. Sixty-four terrestrial gastropods in numerous

ecosystems have been reported to consume lichens worldwide as reviewed by Asplund (2010). For example, gastropods feed on calcicolous lichens in rocky deserts (Shachak *et al.* 1987) and on epiphytic lichens in broadleaved forests (Gauslaa *et al*. 2006).

Gastropod lichenivory has recently been identified as a threat to different foliose epiphytic lichen species, possibly because of increased native and introduced gastropod populations resulting from climatic shifts with increased annual rainfall and milder winters (Sternberg 2000; Willis *et al.* 2006; Environment and Climate Change Canada 2016). For example, gastropod grazing limited the growth and development of juvenile *L. pulmonaria* (Asplund & Gauslaa 2007) and has likely led to declines in populations of *Pseudocyphellaria citrina* (L.) Vain. in coastal deciduous forests of Norway (Gauslaa 2008). The Committee on the Status of Endangered Wildlife in Canada (COSEWIC) has identified introduced gastropod grazing as a threat and research priority for the endangered lichen species *Erioderma pedicellatum* (Hue) P.M. Jørg, in Atlantic Canada (Environment and Climate Change Canada 2018). Other studies in Nova Scotia (Cameron 2009; Environment and Climate Change Canada 2018) and northeastern U.S.A (Clyne *et al.* 2019) have also raised the alarm of the potential threat of introduced gastropod grazing. However, no research has been conducted to evaluate lichen grazing severity in Newfoundland and further resolution is needed to understand how factors such as species diversity affect gastropod grazing severity.

1.4 Native and Invasive Gastropods

We identified eight native and introduced species of gastropods (**Table 1.1**) as probable tree climbing lichenivores in Newfoundland. Four gastropod species included in **Table 1.1** are known to graze lichens. *Arion ater* is a generalist feeder, consuming plant material, carrion, and

other gastropods. *Lehmannia marginata* has a specialized radula that it uses to feed on lichens, mushrooms, and algae (Grimm *et al.* 2009). *Limax maximus* has been documented to consume plant matter, fungi, and lichens in forested or highly vegetated areas (Newell 1967). *Cepaea hortensis* snails have been widely observed grazing lichens in Europe including *L. pulmonaria* (Gauslaa *et al.* 2006).

Table 1.1. List of presumed arboreal gastropod species known to occur on the island of Newfoundland (Moss & Hermanutz 2010; Personal Communication John Maunder)

Gastropod species	Common name	Native/ Introduced	Lichen grazer
Arion ater Linnaeus	Black Arion	Introduced	Yes
Arion distinctus Mabille	Dark-Faced Arion	Introduced	Unknown
Arion fasciatus Nilsson	Orange-Banded Arion	Introduced	Unknown
Cepaea hortensis O. F. Müller	Garden snail	Native	Yes
Cepaea nemoralis Linnaeus	Tree Snail	Introduced	Unknown
Deroceras laeve O. F. Müller	Meadow Slug	Native	Unknown
Lehmannia marginate O. F. Müller	Tree Slug	Introduced	Yes
Limax maximus Linnaeus	Leopard Slug	Introduced	Yes

1.5 Lichen Defense Adaptations

Because many lichens are slow growing and sessile (vs. vagrant lichens), they are vulnerable to overgrazing. Lichens have evolved various defense mechanisms such as low nutritional content, surface toughness, secondary metabolites (e.g., Asplund *et al*. 2010; Fröberg *et al*. 2006; Gauslaa 2005). Some lichen secondary metabolites function as a deterrent to lichenivores (Gauslaa 2005; Asplund *et al*. 2009; Cernajov *et al*. 2014; Wieners *et al*. 2018). Two main groups of lichen metabolites or compounds are produced: primary and secondary metabolites. Primary metabolites are essential for the survival of the lichen (e.g., proteins, amino acids,

carbohydrates) and secondary metabolites are not essential for survival but are often helpful and have various biological roles including defense against lichen grazing, and UV protection (e.g. Gauslaa, 2005a; Lawrey, 1980). For example, some cortical compounds function as solar radiation screens for lichen photobionts against excess light and solar radiation (Nybakken *et al.* 2007).

Lichen secondary metabolites are not equally distributed throughout the thallus (Elix & Stocker-Wörgötter 2008) and different secondary metabolites are restricted to different parts of the thallus and in varying concentrations (Asplund *et al*. 2010). For example, the common secondary metabolite, usnic acid, is limited to the upper cortex in many lichens, or around the spores and the algae in *Cladonia* spp. (Liao *et al.* 2010) where it provides the distinct usnic yellow/green coloring while many colorless depsides and depsidones occur in the medulla (Elix & Stocker-Wörgötter 1996; Dembitsky & Tolstikov 2005; McEvoy *et al.* 2007). *Lobaria pulmonaria* contains seven depsidone compounds of which stictic and norstictic acids are the most predominant. Studies have concluded based on empirical data that these compounds protect certain regions of higher fitness from lichen grazing (McEvoy *et al.* 2007).

1.6 Thesis Objectives

My focus is to establish baseline information for current lichenivory rates and the present gastropod community in Newfoundland as well as to investigate the effect of lichen forage choice and species diversity on lichen grazing severity by gastropods. Establishing a baseline of information that can inform future research is a critical step in identifying if gastropod grazing represents a threat to epiphytic lichen communities.

In Chapter 2, I used field surveys of the lichen and gastropod community to record current lichen grazing levels and describe the lichen and gastropod community within the study area. I used both established methods (e.g., grazing classes, timed searches) and modified methods (i.e., arboreal pitfall traps) to describe lichen grazing and lichen and snail populations within the study area. I compared the effectiveness of these methods and made recommendations for direction of future research.

In Chapter 3 I used two species of tree-climbing snails in lab lichen feeding experiments to examine how forage choice affects lichen consumption with a focus on *L. pulmonaria.* I identified relevant trends in gastropod preference and lichen palatability of three common lichen species in the study area and successfully evaluated whether invasive *C. nemoralis* represents a more aggressive lichenivore compared to the native *C. hortensis.*

My work represents the first evaluation of lichenivory severity in the province of Newfoundland and lays the foundation for continued research. In this thesis I identified important trends in observed lichen grazing and factors influencing grazing severity of ecologically important *L. pulmonaria*. My research will lay the groundwork for more monitoring and research of lichen grazing in Newfoundland and Eastern Canada.

1.7 Co-authorship Statement

This research was supervised by Dr. Michele Piercey-Normore of Memorial University of Newfoundland and Dr. André Arsenault of Canadian Forest Service. As the primary author, I, Katherine Racheal Flores was responsible for the greater construction of this thesis that included contributing the most to the development of the study design and research, data collection, lab experimentation, statistical analysis and writing and revision. Dr. Michele Piercey-Normore and Dr. André Arsenault contributed to the study design, the data collection, lichen identification and writing and revision for Chapter 2, "Gastropod Grazing of Epiphytic Foliose Lichens in a Mixed-Wood Forest of Western Newfoundland" and will be included as co-authors when this chapter is published. Dr. Michele Piercey-Normore and Dr. André Arsenault contributed to the study design, writing and revision for Chapter 3 "Lichen Forage Choice and Gastropod Preference Affect Lichen Biomass Consumed by a Native and Introduced Snail Species" and will be included as co-authors when this chapter is published. I am currently preparing both chapters for submission to the journal, The Lichenologist.

1.8 References

Andersson, L. & Appelqvisit, T. (1987) *Lobaria pulmonaria* and *Gyalecta ulmi* as indicators of deciduous woodland with high nature qualities. *Svensk Botanisk Tidskrift*: 185–194.

Asplund, J. (2010) Lichen-gastropod interaction: Chemical defence and ecological consequences of lichenivory. Norwegian University of Life Sciences.

Asplund, J. & Gauslaa, Y. (2007) Mollusc grazing limits growth and early development of the old forest lichen *Lobaria pulmonaria* in broadleaved deciduous forests. *Oecologia* **155**: 93–99.

Asplund, J. & Wardle, D. A. (2017) How lichens impact on terrestrial community and ecosystem properties. *Biological Reviews* **92**: 1720–1738.

Asplund, J., Solhaug, K.A. & Gauslaa, Y. (2009) Fungal depsidones-an inducible or constitutive defence against herbivores in the lichen *Lobaria pulmonaria*? *Basic and Applied Ecology* **10**: 273–278.

Asplund, J., Solhaug, K. A. & Gauslaa, Y. (2010) Optimal defense: Snails avoid reproductive parts of the lichen *Lobaria scrobiculata* due to internal defense allocation. *Ecology* **91**: 3100–3105.

Baur, B., Fröberg, L. & Baur, A. (1995) Species diversity and grazing damage in a calcicolous lichen community on top of stone walls in Öland, Sweden. *Finnish Zoological and Botanical Publishing Board* **4**(32): 239-250.

Baur, B., Fröberg, L., Baur, A., Guggenheim, R. & Haase, M. (2000) Ultrastructure of snail grazing damage to calcicolous lichens. *Nordic Journal of Botany* **20**: 119–128.

Benesperi, R. & Tretiach, M. (2004) Differential land snail damage to selected species of the

    lichen genus *Peltigera*. *Biochemical Systematics and Ecology* **32**: 127–138.

Benesperi, R., Nascimbene, J., Lazzaro, L., Bianchi, E., Tepsich, A., Longinotti, S. & Giordani,

    P. (2018) Successful conservation of the endangered forest lichen *Lobaria pulmonaria*

    requires knowledge of fine-scale population structure. *Fungal Ecology* **33**: 65–71.

Bolli, J. C., Wagner, H. H., Kalwij, J. M., Werth, S., Cherubini, P., Scheidegger, C. & Rigling,

    A. (2008) Growth dynamics after historic disturbance in a montane forest and its

    implications for an endangered epiphytic lichen. *Botanica Helvetica* **118**: 111–127.

Brez, J. (1791) *La Flore des Insectophiles*. Utrecht: B. Wild et J. Altheer.

Cameron, R. (2009) Are introduced gastropods a threat to endangered lichens? *Canadian Field-*

    Naturalist **123**: 169–171.

Campbell, J. & Fredeen, A. L. (2004) *Lobaria pulmonaria* abundance as an indicator of

    macrolichen diversity in Interior Cedar-Hemlock forests of east-central British Columbia.

    Canadian Journal of Botany **7**(82): 970-982.

Černajová, I., & Svoboda, D (2014) Lichen compounds of common epiphytic Parmeliaceae

    species deter gastropods both in laboratory and in Central European temperate forests.

    Fungal Ecology 11: 8-16.

Černajová, I. & Škaloud, P. (2019) The first survey of Cystobasidiomycete yeasts in the lichen

    genus *Cladonia*; with the description of *Lichenozyma pisutiana* gen. nov., sp. nov.

    Fungal Biology **123**: 625–637.

Clyne, A. B., Cleavitt, N. L. & Fahey, T. J. (2019) Terrestrial gastropod grazing on macrolichens in a Northern broadleaf–conifer forest. *Northeastern Naturalist* **26**: 261-274.

Coker, P. D. (1967) Damage to Lichens by Gastropods. *The Lichenologist* **3**: 428.

Damman, A. W. (1983) An ecological subdivision of the Island of Newfoundland. *Biogeography and Ecology of the Island of Newfoundland* (R. South, ed): pp. 163–172. Springer, Netherlands.

Dembitsky, V. M. & Tolstikov, G. A. (2005) *Organic Metabolites of Lichens*. Novosibirsk Publishing House of SB RAS.

Edman, M., Eriksson, A.-M. & Villard, M.-A. (2007) Effects of selection cutting on the abundance and fertility of indicator lichens *Lobaria pulmonaria* and *Lobaria quercizans*. *Journal of Applied Ecology* **45**: 26–33.

Elix, J. & Stocker-Wörgötter, E. (1996) Biochemistry and secondary metabolites. *Lichen Biology*. (T. H. Nash III, ed): pp. 106-136. Cambridge University Press.

Elix, J. & Stocker-Wörgötter, E. (2008) Biochemistry and secondary metabolites. *Lichen Biology* (T. H. Nash, ed): pp. 104–133. Cambridge: Cambridge University Press.

Ellis, C. J. & Coppins, B. J. (2006) Contrasting functional traits maintain lichen epiphyte diversity in response to climate and autogenic succession. *Journal of Biogeography* **33**: 1643–1656.

Environment and Climate Change Canada (2016) *Climate data and scenarios for Canada: Synthesis of recent observation and modelling results*. Government of Canada, Canada.

Environment and Climate Change Canada (2018) *Action Plan for the Boreal Felt Lichen (*Erioderma pedicellatum*) (Atlantic population) and Vole Ears Lichen (*Erioderma mollissimum*) in Canada [Proposed]*. Ottawa, ON: Canada. Committee on the Status of Endangered Wildlife in Canada.

Environment Canada (2015) South Brook Pasadena Weather Station Annual Climate Data. *Environment Canada*.

Fröberg, L., Baur, A. & Baur, B. (1993) Differential herbivore damage to calcicolous lichens by snails. *The Lichenologist* **25**: 83–95.

Fröberg, L., Baur, A. & Baur, B. (2006) Field study on the regenerative capacity of three calcicolous lichen species damaged by snail grazing. *The Lichenologist* **38**: 491–493.

Gauslaa, Y. (1994) *Lobaria pulmonaria*, an indicator of species-rich forests of long ecological continuity. *Blyttia* **52**: 119–128.

Gauslaa, Y. (1995) The Lobarion, an epiphytic community of ancient forests threatened by acid rain. *The Lichenologist* **27**: 59–76.

Gauslaa, Y. (2005) Lichen palatability depends on investments in herbivore defence. *Oecologia* **143**: 94–105.

Gauslaa, Y. (2008) Mollusc grazing may constrain the ecological niche of the old forest lichen *Pseudocyphellaria crocata*. *Plant Biology* **10**: 711–717.

Gauslaa, Y., Holien, H., Ohlson, M. & Solhøy, T. (2006) Does snail grazing affect growth of the old forest *lichen Lobaria pulmonaria*? *The Lichenologist* **38**: 587–593.

Gerson, U. & Seaward, M. (1977) Lichen-Arthropod Associations. *The Lichenologist* **5**: 434–443.

Goward. T. & Arsenault, A. (2000a) Cyanolichen distribution in young unmanaged forests: a dripzone effect? The Bryologist **103**(1): 28-37.

Goward, T. & Arsenault, A. (2000b) Cyanolichens and conifers: implications for global conservation. *Forest, Snow and Landscape Research* **75**(3): 303–318.

Grimm, W. F., Forsyth, R. G., Schueler, F. W. & Karstad, A. (2010) *Identifying land snails and slugs in Canada: introduced species and native genera*. Canadian Food Inspection Agency.

Hauck, M. (2009) Global warming and alternative causes of decline in arctic-alpine and boreal-montane lichens in north-western Central Europe. *Global Change Biology* **15**: 2653–2661.

Hawksworth, D. L. & Grube, M. (2020) Lichens redefined as complex ecosystems. *New Phytologist* **227**: 1281–1283.

Henderson, A. & Hackett, D. J. (1986) Lichen and algal camouflage and dispersal in the Psocid Nymph *Trichadenotecnum fasciatum*. *The Lichenologist* **18**: 199–200.

Hodkinson, B. P. & Lutzoni, F. (2009) A microbiotic survey of lichen-associated bacteria reveals a new lineage from the Rhizobiales. *Symbiosis* **49**: 163–180.

Johansson, P. (2008) Consequences of disturbance on epiphytic lichens in boreal and near boreal forests. *Biological Conservation* **141**: 1933–1944.

Lawrey, J. D. (1980) Sexual and asexual reproductive patterns in *Parmotrema* (Parmeliaceae) that correlate with latitude. *Bryologist* **1**: 344–350.

Liao, C., Piercey-Normore, M. D., Sorensen, J. L. & Gough, K. (2010) In situ imaging of usnic acid in selected *Cladonia spp*. by vibrational spectroscopy. *Analyst* pp. 3242–3248. Royal Society of Chemistry.

Liira, J. & Sepp, T. (2009) Indicators of structural and habitat natural quality in boreo-nemoral forests along the management gradient. *Annales Botanici Fennici* **46**: 308–325.

McCune, B. & Geiser, L. (2009) *Macrolichens of the Pacific Northwest*. Corvallis: Oregon State University Press.

McEvoy, M., Gauslaa, Y. & Solhaug, K. A. (2007) Changes in pools of depsidones and melanins, and their function, during growth and acclimation under contrasting natural light in the lichen *Lobaria pulmonaria. New Phytologist* **175**: 271–282.

Moss, M. & Hermanutz, L. (2010) Monitoring the small and slimy — Protected areas should be monitoring native and introduced slugs (Mollusca: Gastropoda). *Natural Areas Journal* **30**: 322–327.

Nascimbene, J., Brunialti, G., Ravera, S., Frati, L. & Caniglia, G. (2010) Testing *Lobaria pulmonaria* (L.) Hoffm. as an indicator of lichen conservation importance of Italian forests. *Ecological Indicators* **10**: 353–360.

Nascimbene, J., Casazza, G., Benesperi, R., Catalano, I., Cataldo, D., Grillo, M., Isocrono, D., Matteucci, E., Ongaro, S., Potenza, G., Puntillo, D., Ravera, S., Zedda, L. & Giordani, P.

(2016) Climate change fosters the decline of epiphytic *Lobaria* species in Italy. *Biological Conservation* **201**: 377–384.

Nash III, T. H. (2008) Introduction. *Lichen Biology, second edition*. (T. H. Nash III, ed): pp. 1-8. Cambridge University Press.

Newell, P. (1967) Mollusca. *Soil Biology* (A. Burges & F. Raw, eds): pp. 425–426. London: Academic Press.

Nybakken, L., Asplund, J., Solhaug, K. A. & Gauslaa, Y. (2007) Forest successional stage affects the cortical secondary chemistry of three old forest lichens. *Journal of Chemical Ecology* **33**: 1607–1618.

Pykälä, J. (2004) Effects of new forestry practices on rare epiphytic macrolichens. *Conservation Biology* **18**: 831–838.

Richardson, D. & Cameron, R. (2004) Cyanolichens: Their response to pollution and possible management strategies for their conservation in northeastern North America. *Northeastern Naturalist* **11**: 1–22.

Rose, F. (1974) The Epiphytes of Oak. *The British Oak: Its History and Natural History* (M. M & (F. Perring, eds): pp. 250–273. Classey, Faringdom.

Rose, F. (1988) Phytogeographical and ecological aspects of Lobarion communities in Europe. *Botanical Journal of the Linnean Society* **96**: 69–79.

Scheidegger C. & Walser, J. (1998*) Reintroduction and augmentation of populations of the endangered* Lobaria pulmonaria*: methods and concepts* In: Lobarion Lichens as

Indicators of the Primeval Forests of the Eastern Carpathians. (S. Kondratyuk, (B. Coppins, eds): pp. 33–52. Phytosociocentre.

Scheidegger, C. & Werth, S. (2009) Conservation strategies for lichens: insights from population biology. *Fungal Biology Reviews* **23**: 55–66.

Scheidegger, C., Bilovitz, P. O., Werth, S., Widmer, I. & Mayrhofer, H. (2012) Hitchhiking with forests: Population genetics of the epiphytic lichen *Lobaria pulmonaria* in primeval and managed forests in southeastern Europe. *Ecology and Evolution* **2**: 2223–2240.

Seyd, E. & Seaward, M. (1984) The association of oribatid mites with lichens. *Zoological Journal of the Linnean Society* **80**: 369–420.

Shachak, M., Jones, C. G. & Granot, Y. (1987) Herbivory in rocks and the weathering of a desert. *American Association for the Advancement of Science* **4805**(236): 1098-1099.

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. & McCutcheon, J. P. (2016) Basidiomycete yeasts in the cortex of ascomycete macrolichens. *Science* **353**: 488–492.

Sternberg, M. (2000) Terrestrial gastropods and experimental climate change: A field study in a calcareous grassland. *Ecological Research* **15**: 73–81.

Sundberg, B., Näsholm, T. & Palmqvist, K. (2001) The effect of nitrogen on growth and key thallus components in the two tripartite lichens, *Nephroma arcticum* and *Peltigera aphthosa*. *Plant, Cell and Environment* **24**: 517–527.

Town of Pasadena (2018) *Development Regulations.* Pasadena, NL.

U'Ren, J. M., Lutzoni, F., Miadlikowska, J. & Arnold, A. E. (2010) Community analysis reveals close affinities between endophytic and endolichenic fungi in mosses and lichens. *Microbial Ecology* **60**: 340–353.

Wieners, P. C., Bilger, W. & Gauslaa, Y. (2018) Carbon-based secondary compounds in the lichen *Hypogymnia physodes* deter detrivorous woodlice. *Fungal Ecology* **31**: 54–58.

Willis, J. C., Bohan, D. A., Choi, Y. H., Conrad, K. F. & Semenov, M. A. (2006) Use of an individual-based model to forecast the effect of climate change on the dynamics, abundance, and geographical range of the pest slug *Deroceras reticulatum* in the UK. *Global Change Biology* **12**: 1643–1657.

2. Gastropod Grazing of Epiphytic Foliose Lichens in a Mixed-Wood Forest of Western Newfoundland

2.1 Abstract

Gastropod overgrazing has emerged as a potential threat to some species of epiphytic foliose lichens in Eastern North America and Europe; however, few studies have evaluated this threat, and it is unknown whether gastropod grazing represents a threat to lichen communities in Western Newfoundland. This study investigates how gastropod species currently alter epiphytic foliose lichen communities within a mixed-wood forest of Western Newfoundland by investigating three questions: (1) To what extent has gastropod grazing occurred within the area? (2) What is the tree-climbing gastropod community composition? (3) Does grazing severity vary in relation to tree level environmental factors? We use field grazing severity surveys and lichen abundance and diversity surveys to describe the current state of epiphytic foliose lichens in the study area. We use nocturnal timed searches and arboreal pit-fall traps to describe the abundance of tree-climbing gastropods. Our results show that for select lichen species, including model lichen species *Lobaria pulmonaria*, gastropod grazing is severe to extreme and widespread within the study area. We found that grazing severity level increased with increasing gastropod abundance, and regardless of sampling method, slugs greatly outnumbered snails in the tree-climbing gastropod community. We found that tree-level factors, including lichen location on tree, significantly influenced observed graving severity. This study is the first attempt to evaluate the current state of lichen grazing in Newfoundland and will function as a source of baseline data for further research and monitoring.

2.2 Introduction

Many types of insects, mites and invertebrates make use of lichen thalli for habitat and as a food source (Gerson & Seaward 1977; Seyd & Seaward 1984) without impacting the lichen's vitality; however, gastropod grazing can dramatically alter the composition and distribution of lichen communities (Coker 1967; Asplund & Gauslaa 2007*b*, 2010; Gauslaa 2008; Asplund *et al.* 2010). Gastropod lichenivory has been observed in locations across Europe (Asplund & Gauslaa 2007*b*, 2010; Benesperi & Tretiach 2004; Černajová & Svoboda 2014; Fröberg *et al*., 2011; Gauslaa *et al.* 2006a; Gauslaa 2008) and recent studies have evaluated its presence in North America (Cameron 2009; Clyne *et al.* 2019).

Within the northeastern region of North America, climate change scenarios project increasingly warm and wet weather (Canada 2017), which could make the cool, temperate climate of Newfoundland more conducive to gastropod proliferation. Sternberg (2000) investigated the impact of warm and wet climate change scenario on gastropod populations of calcareous grasslands and reported that climate change led to a significant increase in overall abundance of slugs and snails. Climate change accelerates the introduction and spread of many invasive species while reducing the resilience of ecosystems to biological invasion (Masters & Norgrove 2010). In Nova Scotia, Cameron (2009) found that 90% of arboreal gastropod species surveyed were introduced species to an area where grazing was inferred from observations of damage on the upper cortex and underlying photobiont layer of an endangered cyanolichen species, *Erioderma pedicellatum* (Hue) P.M. Jørg.

Fungi, including lichen mycobionts, represent a source of vast biodiversity and high ecological importance. Regardless, fungi have not been prioritized in the field of conservation

science and remain understudied and under-conserved in comparison to most plants and animals (Stein *et al.* 2000; Allen *et al.* 2019). A major barrier in lichen conservation studies is baseline data deficiency (de Lange *et al.* 2012; Allen *et al.* 2019). Many species of lichens have not been adequately studied to determine whether they are threatened or rare (Molina & Hawksworth 2008; Allen *et al.* 2019). It is therefore paramount to increase the baseline knowledge of all facets of lichen biology to enable effective lichen conservation planning.

Within Newfoundland and in many other localities across Canada, the abundance of native and introduced gastropods, and the extent of introduced gastropod species is confirmed on tree seedlings in forest ecosystems of Newfoundland (Noel 2004; Moss & Hermanutz 2010) there is no research, to our knowledge, on how they affect lichen community dynamics.

As a result of the compounding effects of shifting climatic conditions and increased prevalence of invasive species coupled with preliminary studies indicating that gastropod grazing of lichens may pose a threat, it is important to monitor the potential threat of gastropod grazing. Given the lack of knowledge, we set out to describe the severity of lichen grazing in western Newfoundland with a focus on the lichen species, *Lobaria pulmonaria* (L.) Hoffm. (1796). This study addresses three questions: 1) To what extent has gastropod grazing occurred within the area? (2) What is the tree-climbing gastropod community composition? (3) Does lichenivory vary in relation to tree level environmental factors? We made several predictions related to tree-level factors (**Appendix A.1**). To evaluate severity, we conducted both lichen and lichenivory surveys and used two field methods (one amended, one established) to describe the gastropod community within the area.

2.3 Methods

Study Area. The Blue Gulch Watershed is in the Corner Brook sub-region of the Western Newfoundland ecoregion (49°28.4'N, 57°42.9'W) (Damman 1983). This sub-region features dense mixed-wood boreal forest dominated by *Abies balsamea* (L.) Rich.. The area coincides with the northern limit for tree species *Pinus strobus* L.*, Acer rubrum* L. 1753*,* and *Populus tremuloides* Michx. The sub-region features rugged topography upon a slate and limestone bedrock producing nutrient rich and productive soils (Damman 1983). The climate is wet with an annual mean temperature of 4-7°C and an annual mean precipitation between 800 and 1200 mm (Environment Canada 2015). Although tree cores collected from the area indicate that conifer species were logged recently (approximately 60 years ago), many older remnant *Betula alleghaniensis* Britt. remain in the study area providing a mixed environment of young and older growth. The surveyed area is located at the base of a steep southwestern slope with many small streams and creeks leading down the slope towards the main water bodies.

Experimental Design. We selected 70 survey trees (>10 cm diameter at breast height [DBH]) that hosted *L. pulmonaria* along five transects that were 100 m apart and ran parallel to one another for 800 m from NE-SW parallel to the slope. Survey trees were equally split between conifer and broadleaf trees. Conifer trees included *A. balsamea, Picea mariana* (Mill.) Britton, Sterns & Poggenburg, and *Picea glauca* (Moench) Voss*,* and broadleaf trees included *B. alleghaniensis* and *A. rubrum.*

Lichen survey. We identified all thalli of macro-lichen species present below two meters on all sampling trees. We collected voucher samples for all species, which have been deposited in the Sir Wilfred Grenfell Campus Herbarium of Memorial University of Newfoundland

(SWGC). We recorded lichen abundance for each lichen species (and the most common crustose lichens) on both the bole and the branches of each survey tree using a modified scale (**Table 2.1**) developed by Goward & Arsenault (2000). This method incorporates both a count and an abundance scale to resolve the issues in surveying lichen species of varying thallus size, or those which occupy three-dimensional space (Stevenson & Enns 1993; Peterson & McCune 2001). Within this scale, colonies are defined as a group of lichen thalli of the same species (from one-to-many individuals), which were once independent but have grown into a network of thalli.

Table 2.1. Lichen abundance scale (Goward & Arsenault 2000)

Unit	Description
1	2 or fewer colonies on branches/bole of tree
2	3-5 colonies on branches/bole of tree
3	6 colonies on branches/bole of tree or less than 20% cover.
4	7-10 colonies on branches/bole of tree or 21-50% cover
5	11+ colonies on branches/bole or greater than 51% cover

Lichenivory survey. We adapted the methods of Clyne et al. (2019) to describe lichenivory (**Fig 2.1**) by including the addition of a more extreme lichenivory class, and by merging of the two lowest classes, as well as adding details within the existing lichenivory classes. We recorded all lichen species present from ground level to two meters and rated them based on the extent of lichenivory on the thallus surface. We surveyed lichens present on boles and on branches separately and used a hand lens (10X) to perform a visual scan of the lichen thalli.

Figure 2.1. Amended lichenivory classes from Clyne et al. (2019). Class 1 = grazing marks visible for 1 or more thalli with 10X magnification lens only, class 2 = grazing damage visible with the 10X magnification lens and the naked eye on 1 or more thalli, class 3 = grazing damage obvious and extensive on most or all thalli with the naked eye, and class 4 = grazing so extensive thalli appear skeletal with the naked eye.

Nocturnal timed surveys. We aimed to perform searches on survey trees on warm (10-15 °C) and moist nights after or during rain, between 9 pm and 12 am. We examined all surface area of the tree boles up to two meters above ground using headlamps for three minutes. Within this time, we recorded all gastropods located on the tree bole by group (i.e., slug or snail) where slug refers to an animal without an external shell and snail refers to an animal with a shell (Ponder *et al.* 2019). Each survey tree was searched once within the period of August 1st - August 28th of 2020.

Arboreal pit-fall traps. To further describe the gastropod community, we used arboreal pit-fall traps to collect specimens (**Fig. 2.2**). Ground pitfall traps are a common and effective method of sampling terrestrial gastropod populations (Mccoy 1999; Lucid *et al.* 2018). Arboreal pitfall traps are used less commonly but have been used to sample cursorial spiders on tree boles (Pinzón & Spence 2008). Traps installed on all survey trees consisted of a plastic cup with drain holes on the upper sides stapled to the tree bole approximately one meter from ground level. Traps were set-up at this height for ease of collection and were baited with diluted propylene glycol to help preserve specimens. Although it is not known if propylene glycol is attractant to gastropods, it has a faintly sweet taste. Pitfall traps were set up for approximately 2 weeks from August 15th - August 30th. Collected specimens were sorted into gastropods and non-gastropods and stored in 90% ethanol.

Figure 2.2. Arboreal pit-fall trap on a *B. alleghaniensis* survey tree in the Blue Gulch Watershed of Pasadena, Newfoundland.

     Statistical Analysis. The POLR function of the MASS package (Venables & Ripley 2002) in R 4.0.0 (R Core Team 2019) was used for all analyses and the ggplot2 package (Wickham, 2016) and effects package (Foc & Hong 2009) was used to create figures. We used proportional odds logistic regression models (with α threshold = 0.05) to test whether lichenivory class distribution (**Fig 2.1**) was a function of gastropod presence metrics. To determine whether lichenivory varies in relation to tree level environmental factors, we compiled a list of working hypotheses to describe patterns in lichen grazing (**Appendix A.1**). To determine which environmental factors should be included in the model, an information-theoretic approach (Anderson 2008) was used to rank competing models using Akaike Information Criterion (AIC) (Burnham & Anderson 2002). Pearson correlations were used to test the relationship among

environmental variables. Models were tested for assumptions and fit, proportional distribution

and pretending variables following Leroux (2019). To assess how well the models described the

data, the β coefficient, Standard Error (SE) and the significant value (p-value) were extracted

(with α threshold = 0.05). The MuMln (Barton 2020) package was used to model selection and

comparison based on AIC values.

The model form we used was $GCa = e^{1/n} + $Gamma *error*, where *GCa* = Grazing class of

each lichen species per survey tree (Class 1-4), n = number of lichens surveyed, and Gamma

error refers to the error in the normal approximation to the gamma distribution. Both data subsets

(all macro-lichen species including *L. pulmonaria*) were used to test if data from the timed

gastropod searches and the pitfall gastropod traps explained the observed patterns of grazing.

Top tree level environmental predictor factors were included in the models to allow for the best

fit (**Appendix A.1**).

2.4 Results

Patterns of gastropod grazing damage

We rated 70 % of thalli (108 thalli on 70 trees) as severely or extremely affected by lichenivory (**Table 2.2**, **Fig. 2.4**), and only 24% were minimally grazed. *Lobaria pulmonaria* was the only lichen species which was represented in the severe to extreme grazing classes and all other species were represented in the minimum to moderate grazing classes (**Table 2.2**). The representation of all lichen species other than *L. pulmonaria* in lichenivory classes above "minimal" was so sparse that they could not be included in a logistical regression to determine significance (**Table 2.2**). *L. pulmonaria,* was represented in all grazing classes but was most abundant in the severe and extreme classes showing a positive trend in association with grazing class.

Table 2.2. Lichenivory class percentage per species and number of thalli in parenthesis for foliose epiphytic lichens and common crust lichens on conifer (con.) and broadleaved (broad.) trees in Blue Gulch Watershed, NL. Refer to Fig 2.1 for grazing classes. Asterix (*) indicates common crust lichens.

Lichen	Lichen thalli rated	On con.	On broad.	Lichenivory class percentage			
				Min.	Mod.	Severe	Extreme
Alectoria sarmentosa (Ach.) Ach.	3	1	0	100(3)	0	0	0
Cladonia squamosa Hoffm.	2	0	2	100(1)	0	0	0
Hypogymnia incurvoides Rass.	4	4	0	100(4)	0	0	0
Hypogymnia physodes (L.) Nyl.	2	2	0	100(2)	0	0	0
Lobaria pulmonaria (L.) Hoffm.	93	57	36	2(2)	3(3)	76(70)	19(18)
Lobaria quercizans Michx.	1	0	1	100(1)	0	0	0
Lobaria scrobiculata (Scop.) DC.	16	5	11	13(2)	6(1)	81(13)	0
Melanelia subaurifera (Nyl.) Essl.	1	1	0	100(1)	0	0	0
Parmelia squarrosa Hale	27	18	9	67(18)	70(2)	40(1)	22(6)
Parmelia sulcata Taylor	1	1	0	100(1)	0	0	0
Platismatia glauca (L.) Culb. & C. Culb.	5	5	0	60(3)	40(2)	0	0
Graphis scripta (L.) Ach. *	4	0	4	100(4)	0	0	0
Lepraria lobificans Nyl. *	4	0	1	100(4)	0	0	0
Loxospora ochrophaea (Tuck.) R.C. Harris*	1	1	0	100(1)	0	0	0
Mycoblastus sanguinarius (L.) Norman*	3	3	0	100(3)	0	0	0
Thelotrema lepadinum (Ach.) Ach*	6	0	1	100(6)	0	0	0
All lichens	**154**	**93**	**59**	**24(37)**	**5(8)**	**54(84)**	**44(24)**

Effect of tree level environmental factors on grazing

Lichen grazing class was best described by the location of lichen thalli and the lichen species diversity on the survey tree (**Weight = 0.98; Residual deviance = 239.15; R^2 = 0.57; LR = 109.28**) (**Fig 2.3**; **Appendix A.2; A.3**). Location (bole vs branch) of thalli was a significant predictor of grazing level (**β = -1.86; SE = 0.45; p < 0.01**). Lichen thalli located on boles were more likely to experience severe and extreme grazing while lichen thalli on branches were likely to experience low and moderate grazing. Lichen species diversity was a significant predictor of grazing class (**β = -0.55; SE = 0.25; p =0.029**) and showed a negative trend where lichenivory increased with decreasing species diversity on the survey tree. However, the sample number for lichen diversity was not high enough for species other than *L. pulmonaria* to draw any conclusions. Although not included in the best fitting model, we found that tree type (conifer or broadleaf) significantly predicted the lichenivory class (**β = -1.01; SE = 0.33; p < 0.00**) when we considered all surveyed lichen.
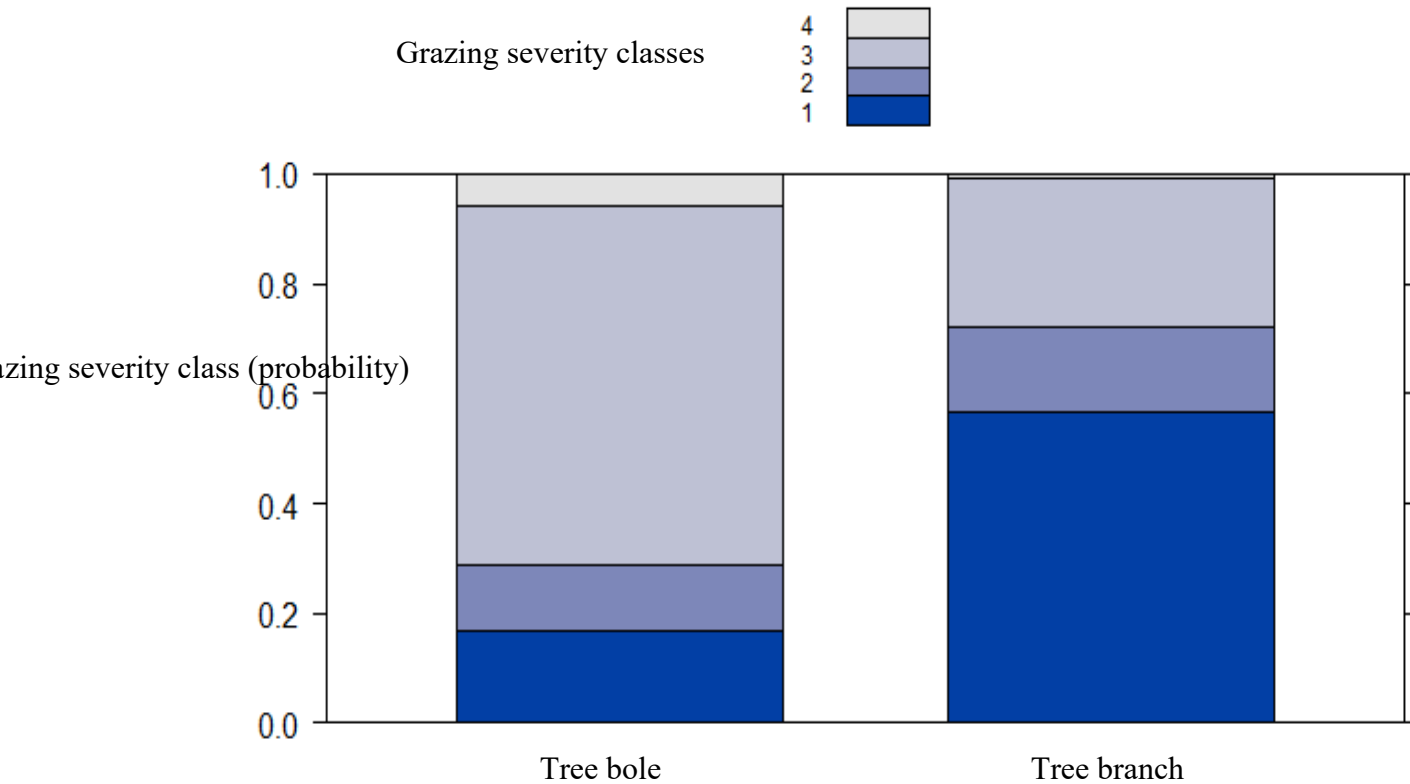
Figure 2.3. Lichen grazing severity class distribution for tree bole and tree branch. Grazing class increased from minimum grazing to extreme grazing from dark blue to light blue, where dark blue represents minimum grazing class, and the lightest blue represents the extreme lichenivory class.

Tree-climbing gastropod community

Within the study area, lichenivory increased with greater gastropod abundance. There was a significant positive relationship between the number of gastropods sampled from arboreal pit-fall traps and lichenivory for all lichen species (**β = 0.46; SE = 0.13; p < 0.05**) and the *L. pulmonaria* data subset (**β = 0.57; S= 0.17; p <0.050; Table 2.2; Fig 2.4**). Gastropod counts from timed searches were not a significant predictor of grazing class for all lichens (**β = -0.05; SE = 0.08; p = 0.53**), nor the *L. pulmonaria* data subset (**β = 0.16; SE = 0.11; p = 0.14; Table**

2.4). Overall, the pit-fall traps collected a total of 177 gastropods (142 slugs and 35 snails) over a period of approximately 2 weeks. The 3-minute timed searches conducted on all survey trees yielded a total count of 98 gastropod individuals (92 slugs and 6 snails). Regardless of sampling method, slugs greatly outnumbered snails in the observed arboreal gastropod community. Five gastropod species were identified in the arboreal pitfall traps (**Table 2.3**), including three species of slug and two species of snail.

Table 2.3. Gastropod species identified from arboreal pit-fall traps where species names with cf. (confer) indicates that it is likely that species, but the identification is not definitive.

Gastropod species	Number on conifer	Number on broadleaf	Total number
Arion hortensis A. Férussac	6	21	26
Arion cf. hortensis	1	0	1
Deroceras laeve O. F. Müller	4	3	6
Deroceras cf. laeve	0	1	1
Discus whitneyi Newcomb	0	4	4
Euconulus fulvus O. F. Müller	0	3	3
Lehmannia marginata O. F. Müller	26	38	63
Lehmannia cf. marginata	0	1	1

Table 2.4. Diagnostic statistics for best fitting lichenivory class predictive models (AIC) using both nocturnal timed gastropod searches and arboreal pitfall traps. LP = L. pulmonaria data subset and All = full data set including all lichen species.

	R^2		Deviance		Likelihood ratio		P-value	
	LP	All	LP	All	LP	All	LP	All
Timed search	0.23	0.61	116.97	235.98	17.89	120.92	0.14	0.59
Pitfall Traps	0.36	0.66	104.98	219.39	29.87	137.52	<0.05	<0.05
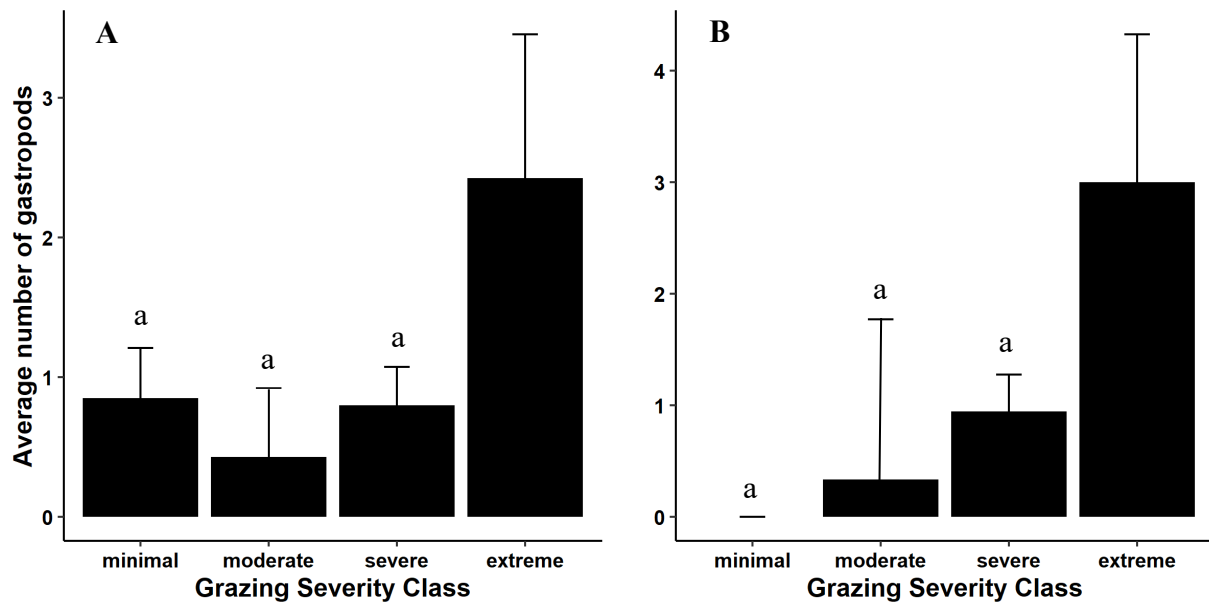
Figure 2.4. Average number of gastropods sampled from arboreal pitfall traps per tree ± standard error compared to lichen grazing severity (classes 1-4) for A) all lichen species, and B) *Lobaria pulmonaria*. Error bars marked with different letters are significantly different.

2.5 Discussion

Lichenivorous gastropods have the capacity to shape epiphytic lichen communities (Asplund & Gauslaa 2007*b*; Gauslaa 2008; Asplund *et al.* 2010). Asplund & Gauslaa (2007) found that lichenivorous gastropods are a threat to survival of juvenile thalli of the threatened lichen species, *L. pulmonaria,* in Norway. Gastropod lichenivory was suggested a major threat and research priority for the COSEWIC-listed endangered cyanolichen species, *Erioderma pedicellatum* in Nova Scotia (Environment and Climate Change Canada 2018). Within the northeastern region of North America, climate change is projected to lead to increasingly warm and wet weather (Canada 2017), which is hypothesized to lead to greater slug and snail abundance (Sternberg 2000**).** Climate change facilitates the introduction and spread of many invasive specie**s,** while simultaneously weakening ecosystems to biological attack (Masters & Norgrove 2010). It is probable that lichenivorous gastropods will be a greater threat to *L. pulmonaria* in northeastern North America in the future. The compounding effects of climate change, and invasive species provides rationale for the monitoring of gastropod lichenivory. The lack of research on gastropod lichenivory represents a barrier to the effective conservation of lichen species. To mitigate the emerging threat of gastropod grazing to foliose tree-lichens it is critically important to start proactive monitoring before the species have declined past the point of return.

We found that gastropod grazing had a significant impact on the epiphytic lichen communities on lower boles within our study areas in western Newfoundland. Overall, lichenivory on cephalolichens was severe to extreme, and widespread within the study area. Extreme grazing was described as grazing so extensive that thalli appeared skeletal (**Fig 2.1)** meaning that the upper cortex, photobiont, medulla, and lower cortex layers of the lichen thallus

45

have been consumed in places with only the ridges left in place. Few studies have investigated gastropod lichenivory in North America; however, our findings are like preliminary observations of extensive lichenivory on endangered lichen species *E. pedicellatum* in Nova Scotia (Cameron 2009). In a study conducted in northeastern U.S.A. which used similar grazing class metrics to this study, Clyne *et al*. (2019) described grazing as minimal to moderate. Although we did not investigate how different lichenivory classes translate in terms of decreased growth and reproduction, the level of grazing observed within the study area is severe and deserves further monitoring to evaluate population-level threats and potential conservation actions.

Lobaria pulmonaria experienced the most severe grazing

We found that the amount of lichenivory was not consistent for all species of lichens surveyed. Different species of lichens experienced varying amounts of grazing. *L pulmonaria* experienced dramatically more grazing both in comparison to a species in the same genus, *L. quercizans*, and species in different genera such as *P. squarros*a and *H. incurvoides* (**Table 2.3**). Previous studies have quantified grazing of *L. pulmonaria,* but none have described such severe lichenivory. Clyne *et al*. (2019) described *L. pulmonaria* grazing as moderate (class 2) and Gauslaa *et al*. (2006b) found that frequent snail lichenivory was not detrimental to the growth of mature *L. pulmonaria* thalli. However, snail lichenivory was detrimental to the development of juvenile *L. pulmonaria* (Asplund & Gauslaa 2007*b*) because the secondary metabolites produced to deter lichenivory increase as the lichen ages and grows (Asplund & Gauslaa 2007*a*). Lichen palatability is influenced by a variety of factors as reviewed by Rundel (1978) including the production of certain secondary metabolites (Gauslaa 2005). Secondary metabolite concentration varies considerably in *L. pulmonaria* depending on soil pH, altitude (Vatne *et al.* 2011), thallus age (Asplund & Gauslaa 2007*a*) and region. Within North America, gastropods discriminate

between two chemical races of *L. pulmonaria* containing different secondary metabolites (Culberson 1969; Asplund 2011). Three distinct genetic lineages of *L. pulmonaria* have been identified between samples from Newfoundland and British Columbia, Canada and Taarenwald, Switzerland (Walser *et al.* 2003; Per. Com. Jessica L. Allen). If a chemical race and/or the genetic lineage of *L. pulmonaria* in western Newfoundland produces fewer grazing-deterring secondary metabolites, and/or produce them in lower concentrations, it would provide a reason for the high levels of lichen grazing in our study area.

In response to potential defense mechanisms (i.e., secondary metabolites, surface toughness, low nutritional value) of lichens, lichenivores gastropods have evolved adaptations to overcome the defenses. *Lobaria pulmonaria* was frequent within the study area and present on every survey tree according to our experimental design. In a study using crustose lichens, Boch *et al*. (2015) hypothesize that gastropods prefer frequent lichens and avoid less frequent lichens based on lichen secondary metabolite recognition. This allows gastropods to adapt to the most abundant food source by developing mechanisms to process lichen secondary metabolites (Boch *et al.* 2015). Boch *et al.* (2015) found that some secondary metabolites in frequent crustose lichen species stimulated snail feeding, while some secondary metabolites in infrequent crustose lichens deterred feeding indicating that snails used secondary metabolite recognition to select forage. Although we did not observe any grazing on crustose lichen species, we found that the most frequently surveyed lichen faced the greatest lichenivory. However, it is not possible to say that *L. pulmonaria* was the most frequent species in the area. Future research is needed to determine if the secondary metabolites found in *L. pulmonaria* in western Newfoundland deter or stimulate gastropod grazing.

Our findings underscore *L. pulmonaria* as a species which requires further monitoring within Western Newfoundland. Although *L. pulmonaria* is common within portions of Newfoundland and, more broadly, the coastal regions of Canada, populations of this species are in decline throughout Europe (Asplund & Gauslaa, 2007b; Nascimbene *et al.* 2016; Juriado *et al.* 2010) and regions of the U.S.A. (Clyne *et al.* 2019). In line with Clyne *et al.* (2019), we recommend further monitoring of lichenivory of *L. pulmonaria*.

Patterns of grazing are shaped by tree-level environmental factors

Our study found that some patterns in lichen grazing were explained by tree-level environmental factors. The most influential environmental factor in this study was thallus location on tree (bole or branch). Lichen thalli were more severely grazed when they were located on tree boles. One explanation for this is the increased accessibility of tree boles compared to tree branches for tree-climbing gastropods, specifically slugs who may need to leave the tree every morning to avoid the sun if sufficient shelter cannot be found within the canopy. If gastropods move from the ground onto the tree to graze lichens, tree boles represent a much shorter distance compared to traversing onto the tree branches. In a study that investigated the vertical distribution of epiphytic lichens in tree canopies, Asplund *et al.* (2010) found that grazing pressure decreased as distance from the ground increased. The study concluded that gastropods help determine the distribution and spatial patterns of epiphytic lichens along the vertical canopy gradient (Asplund *et al.* 2010).

We found that grazing severity depended on tree type (conifer or broadleaf) where lichenivory was significantly less severe on conifer trees compared to broadleaved trees. Our findings support those of Clyne et al. (2019) who found that tree type was the best predictor of gastropod grazing severity in a northern broadleaf-conifer forest and Asplund et al. (2018), who

found that gastropod grazing of epiphytic lichen communities in Norway depended on tree species.

Slugs, not snails, make up most of the arboreal gastropod community

Within the study area, both gastropod sampling methods showed that the population of slugs far outnumbered that of snails implying that slugs may be the more prominent lichenivores. *Lehmannia marginata* and *Arion hortensis* were the most common slug species identified in arboreal pit-fall traps. Both common slugs are introduced species to Canada (Moss & Hermanutz 2010; Personal Communication John Maunder). These results align with those of Cameron (2009) who found only slug species when surveying epiphytic macro-lichen communities in Nova Scotia for gastropod grazing. In contrast, in surveys done in the northeastern U.S.A., Clyne *et al*. (2019) found that snails, not slugs were more abundant in both biomass and individuals. Many factors could affect community composition of tree-climbing gastropods. For example, snails require considerable amounts of calcium for reproduction (Crowell 1973; Wareborn 1979), growth (Gomot *et al.* 1989; Ireland 1991), and shell production (Russell-Hunter 1983; Fournié & Chétail 1984). Calcium availability was the most important factor limiting snail populations in a study conducted on a deciduous forest in the northern U.S.A (Skeldon *et al.* 2007). It is possible that our study area did not provide adequate calcium levels to foster a large population of snails. Another possibility is that our surveys occurred during periods of slug activity but not snail activity. Terrestrial gastropod locomotion is restricted to daily periods of favorable light, moisture, and temperature conditions. Depending on the species, temperature and light are argued to be the most important factors controlling slug locomotion (Dainton 1943, 1954*a*, *b*; Karlin 1961). Cameron (1970) found that snail species *Cepaea hortensis* O. F. Müller and *Cepaea nemoralis* Linnaeus were active at the darkest part of the night and into the early

morning. Our surveys occurred just after sundown and extended until around midnight. This could have ruled out snail species (or slug species) that are more active in the very early morning, as opposed to early night.

Timed searches and pit-fall traps are common and effective methods used to quantify and collect gastropods (Lucid *et al.* 2018), but these traps and searches are traditionally set within the ground among the tree litter. Quantification of litter-based gastropods is not a reliable method to quantify tree-climbing gastropods (Vatne *et al.* 2010). In this study, we compared the effectiveness of arboreal pit-fall traps (**Fig 2.2**) and timed searches. Both methods have their strengths and weaknesses, pit-fall traps are easily set up and allow for collection over a longer time period, but they are not effective in retaining all organisms collected or preventing scavengers from consuming collected organisms. Timed searches are quick (because they are time-limited and driven by effort) but are limited to the expertise of the searcher and are over a short period of time with low effort often yielding low numbers. Timed searches are also less accessible since they are most effective on wet nights when field work is the least practical because of dark and poor conditions. The arboreal pit-fall traps yielded a greater number of gastropod specimens compared to the timed searches. This is not unexpected given the difference in effort between the methods. Timed searches occurred once at each tree for 3-minutes while the arboreal pit-fall traps were deployed for two weeks.

The summer of 2020 was unusually dry in western Newfoundland, and although timed searches should have optimally been performed on warm (10-15 degrees Celsius) and wet (after or during rain) nights, this was not always possible because of COVID-19 restrictions. This emphasizes the difficulty of standardizing for changing weather during timed searches and the compounding effects of an unprecedented global event on the already unique operational

challenges of conducting time- and weather-sensitive field work. Gastropods caught in the arboreal pit-fall traps tended to be very small in size in comparison to those observed actively grazing lichens during the nocturnal timed searches. Due to the low density and discrepancies between observed gastropods in the two methods, we believe that the arboreal pit-fall traps may have been inadequate in catching larger gastropod specimens, while the timed searches may not have fully captured the diversity and abundance of smaller invertebrates. Through combining these two methods, we were able to maximize the diversity of gastropods sampled; however, the arboreal gastropod population may have been further described with increased surveying throughout the entire warm season when gastropods are active.

2.6 Conclusions and Future Recommendations

In this study, we found that gastropod grazing is severe to extreme and common on cephalolichens in a mixed-wood forest of western Newfoundland and lichenivory increased with increasing gastropod abundance. We found that grazing severity is lichen species specific, and *L. pulmonaria* experienced the greatest gastropod grazing. We have shown that different lichen species experience different lichenivory pressure in the forested environment and therefore grazing gastropods change the composition of the epiphytic vegetation. We found that patterns of lichenivory are shaped by variables at the tree-level. Specifically, that lichen accessibility to gastropods is an important factor driving lichenivory extent for the overall lichen community. We identified two of the most common slug species in the tree- climbing gastropod community. We recommend that more sites in Newfoundland be surveyed across a longer temporal frame to improve resolution of the extent and severity of lichenivory both seasonally and regionally.

Our study is the first to describe the current state of gastropod lichenivory in the province of Newfoundland and Labrador and will function as a source of baseline data for further research and monitoring. Further research should focus on providing further validation of the grazing patterns that exist such as whether grazing was due to preference or avoidance of certain lichen species and if that preference or avoidance correlates with lichen secondary metabolite content and concentration. Further research is needed to identify if the level of lichenivory sustained on *L. pulmonaria* could impact survival, growth, and reproduction.

2.7 References

Allen, J. L., McMullin, R. T., Tripp, E. A. & Lendemer, J. C. (2019) Lichen conservation in North America: a review of current practices and research in Canada and the United States. *Biodiversity and Conservation* **28**: 3103–3138.

Anderson, D. R. 2008. Model based inference in the life sciences, a primer on evidence. Springer-Verlag New York, U.S.A.

Asplund, J. (2011) Chemical races of *Lobaria pulmonaria* differ in palatability to gastropods. *The Lichenologist* **43**: 491–494.

Asplund, J. & Gauslaa, Y. (2007*a*) Content of secondary compounds depends on thallus size in the foliose lichen *Lobaria pulmonaria*. *The Lichenologist* **39**: 273–278.

Asplund, J. & Gauslaa, Y. (2007*b*) Mollusc grazing limits growth and early development of the old forest lichen *Lobaria pulmonaria* in broadleaved deciduous forests. *Oecologia* **155**: 93–99.

Asplund, J. & Gauslaa, Y. (2010) Grazing damage in the old forest lichen *Lobaria pulmonaria* increases with gastropod abundance in deciduous forests. *The Lichenologist* **42**(5): 615-619.

Asplund, J., Larsson, P., Vatne, S. & Gauslaa, Y. (2010) Gastropod grazing shapes the vertical distribution of epiphytic lichens in forest canopies. *Journal of Ecology* **98**: 218–225.

Barton, K. (2020) Multi-Model Inferences. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.r-project.org/.

Benesperi, R., Tretiach, M. (2004) Differential land snail damage to selected species of the lichen genus *Peltigera*. *Biochemical Systematics and Ecology* **32**:127-138

Boch, S., Fischer, M. & Prati, D. (2015) To eat or not to eat—relationship of lichen herbivory by snails with secondary compounds and field frequency of lichens. *Journal of Plant Ecology* **8**: 642–650.

Environment and Climate Change Canada (2018) *Recovery Strategy for the Boreal Felt Lichen (Erioderma pedicellatum), Atlantic Population, in Canada*. Ottawa, ON.

Burnham, K. & Anderson, D. (2002) *Model selection and multimodel inference: a practical information theoretic approach*. New York: Springer New York. U.S.A.

Cameron, R. A. (1970) The effect of temperature on the activity of three species of helicid snail (Mollusca: Gastropoda). *Journal of Zoology* **162**: 303-315.

Cameron, R. (2009) Are introduced gastropods a threat to endangered lichens? *Canadian Field-Naturalist* **123**: 169–171.

Černajová, I., Svoboda, D. (2014) Lichen compounds of common epiphytic Parmeliaceae species
deter gastropods both in laboratory and in Central European temperate forests. *Fungal
Ecology* **11**: 8-16.

Clyne, A. B., Cleavitt, N. L. & Fahey, T. J. (2019) Terrestrial gastropod grazing on macrolichens
in a northern broadleaf–conifer forest. *Northeastern Naturalist* **26**(2): 261-274.

Coker, P. D. (1967) Damage to lichens by gastropods. *The Lichenologist* **3**: 428.

Crowell, H. H. (1973) Laboratory study of calcium requirements of the brown snail, *Helix
aspersa* Muller. *Proceedings from the Malacological Society of London* pp. 491–503.

Cruz, A. (1977) Ecology and behavior of the Jamaican woodpecker. *Bulletin of the Florida State
Museum (Biological Sciences)* **22**: 7–8.

Culberson, C. F. (1969) Chemical studies in the genus *Lobaria* and the occurrence of a new
tridepside, 4-O-methylgyrophoric acid. *The Bryologist* **72**: 19.

Dainton, B. H. (1943) Effect of air currents, light, humidity, and temperature on slugs. *Nature*:
25–26.

Dainton, B. H. (1954*a*) The activity of slugs. I. The introduction of activity by changing
temperature. *Journal of Experimental Biology*: 165–187.

Dainton, B. H. (1954*b*) The activity of slugs. II. The effect of light and air currents. *Journal of
Experimental Biology*: 188–197.

Damman, A. W. (1983) An ecological subdivision of the Island of Newfoundland. In:
Biogeography and Ecology of the Island of Newfoundland (R. South, ed): pp. 163–172.
Springer Netherlands.

Environment and Climate Change Canada (2017) *Climate data and scenarios: synthesis of recent observation and modelling results*, Ottawa, ON, Canada.

Environment Canada (2015) South Brook Pasadena Weather Annual Climate Data. *Community Accounts: Climate Data*. Ottawa. ON, Canada.

Foc, J. & Hong, J. (2009) Effect displays in R for multinomial and proportional-odds logit models: Extensions to the effects package. *Journal of Statistical Software* **8**(15): 1-27.

Fournié, J. & Chétail, M. (1984) Calcium dynamics in land gastropods. *American Zoologist* **24**: 857–870.

Fröberg. L., Stoll, P., Baur, A., Baur, B. (2011) Snail herbivory decreases cyanobacterial abundance and lichen diversity along crack of limestone pavements. *Ecosphere* **38**: 1-43.

Gauslaa, Y. (2005) Lichen palatability depends on investments in herbivore defence. *Oecologia* **143**: 94–105.

Gauslaa, Y. (2008) Mollusc grazing may constrain the ecological niche of the old forest lichen *Pseudocyphellaria crocata*. *Plant Biology* **10**: 711–717.

Gauslaa, Y., Holien, H., Ohlson, M. & Solhøy, T. (2006) Does snail grazing affect growth of the old forest lichen *Lobaria pulmonaria*? *The Lichenologist* **38**: 587–593.

Gerson, U. & Seaward, M. (1977) Lichen-arthropod associations. *The Lichenologist* **5**: 434–443.

Gomot, A., Gomot, L., Boukraa, S. & Bruckert, S. (1989) Influence of soil on the growth of the land snail *Helix aspersa*: an experimental study of the absorption route for the stimulating factors. *Journal of Molluscan Studies* **1**(55): 1–7.

Goward, T., Arsenault, A. (2000) Cyanolichen distribution in young unmanaged forests: A dripzone effect? *The Bryologist* **103**(1): 28-37.

Hinds, J. & Hinds, P. (2007) *The macrolichens of New England: descriptions keys, conservation status*. New York: New York Botanical Garden Press.

Ireland, M. P. (1991) The effects of dietary calcium on growth, shell thickness, and tissue calcium in the snail *Achatina fulica*. *Comparative Biochemistry and Physiology* **98**: 111–116.

Jüriado, I. & Liira, J. (2010) Threatened forest lichen *Lobaria pulmonaria* - its past, present, and future in Estonia. *Forestry Studies* **53**: 15–24.

Karlin, E. J. (1961) Temperature and light as factors affecting the locomotion activity of slugs. *Nautilus* **74:** 125–130.

de Lange, P., Galloway, D., Blanchon, D., Knight, A., Rolfe, J., Crowcroft, G. & Hitchmough, R. (2012) Conservation status of New Zealand lichens. *New Zealand Journal of Botany* **50**: 303–363.

Leroux S. J. (2019) On the prevalence of uninformative parameters in statistical models applying model selection in applied ecology. *PLOS ONE*. **14**(2): 1-12.

Lucid, M. K., Ehlers, S., Robinson, L. & Cushman, S. A. (2018) Beer, brains, and brawn as tools to describe terrestrial gastropod species richness on a montane landscape. *Ecosphere* **12**(9): e02535. 10.1002/ecs2.2535

Masters, G. & Norgrove, L. (2010) *Knowledge for Life: Climate Change and Invasive Alien Species*. CABI Working Paper. U.S.A.

Mccoy, K. D. (1999) Sampling terrestrial gastropod communities: Using estimates of species

richness and diversity to compare two methods. *Malacologia* **41**(1): 271-281.

Molina, R. & Hawksworth, D. L. (2008) Protecting rare, little known, old-growth forest-

associated fungi in the Pacific Northwest USA: A case study in fungal conservation.

Mycological Research **112**: 613–638.

Moss, M. & Hermanutz, L. (2010) Monitoring the small and slimy — Protected areas should be

monitoring native and introduced slugs (Mollusca: Gastropoda). *Natural Areas Journal*

30: 322–327.

Nascimbene, J., Casazza, G., Benesperi, R., Catalano, I., Cataldo, D., Grillo, M., Isocrono, D.,

Matteucci, E., Ongaro, S., Potenza, G., Puntillo, D., Ravera, S., Zedda, L. & Giordani, P.

(2016) Climate change fosters the decline of epiphytic *Lobaria* species in Italy.

Biological Conservation **201**: 377–384.

Noel, L. (2004) Effects of management and disturbance regimes on early life history processes of

St. John's balsam fir (*Abies balsamea* (L.) Mill.). Memorial University of Newfoundland,

St. John's, Canada.

Peterson, E. B. & McCune, B. (2001) Diversity and succession of epiphytic macrolichen

communities in low-elevation managed conifer forests in Western Oregon. *Journal of*

Vegetation Science **4**: 511-124

Pinzón, J. & Spence, J. (2008) Performance of two arboreal pitfall trap designs in sampling

cursorial spiders from tree trunks. *Journal of Arachnology* **36**: 280–286.

Ponder, W. F., Lindberg, D. R. & Ponder, J. M. (2019) Shell, body, and muscles. *Biology and Evolution of the Mollusca* pp. 55–132. CRC Press. Boca Raton, U.S.A.

R Core Team. 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.r-project.org/.

Rundel, P. W. (1978) The ecological role of secondary lichen substances. *Biochemical Systematics and Ecology* **6**: 157-170.

Russell-Hunter, W. D. (1983) *The Mollusca*. New York: Academic Press.

Seyd, E. & Seaward, M. (1984) The association of oribatid mites with lichens. *Zoological Journal of the Linnean Society* **80**: 369–420.

Skeldon, M. A., Vadeboncoeur, M. A., Hamburg, S. P. & Blum, J. D. (2007) Terrestrial gastropod responses to an ecosystem-level calcium manipulation in a northern hardwood forest. *Canadian Journal of Zoology* **85**: 994–1007.

Stein, B. A., Adams, J. S. & Hammerson, G. A. (2000) A remarkable array: species diversity in the United States. In: *Precious heritage: the status of biodiversity* (B. A. Stein, (L. S. Kutner & (J. S. Adams, eds): pp. 55–92. New York: Oxford University Press.

Sternberg, M. (2000) Terrestrial gastropods and experimental climate change: A field study in a calcareous grassland. *Ecological Research* **15**: 73–81.

Stevenson, S. K. & Enns, K. A. (1993) *Quantifying arboreal lichens for habitat management: A review of methods*. Nanaimo, B.C. Woodlands Service Division.

Vatne, S., Asplund, J. & Gauslaa, Y. (2011) Contents of carbon based defence compounds in the

old forest lichen *Lobaria pulmonaria* vary along environmental gradients. *Fungal*

Ecology **4**: 350–355.

Vatne, S., Solhøy, T., Asplund, J., Gauslaa, Y. (2010) Grazing damage in the old forest lichen

Lobaria pulmonaria increases with gastropod abundance in deciduous forests. *The*

Lichenologist **42**:615-619.

Venables, W. N. & Ripley, B. D. (2002) Modern Applied Statisitics. Fourth Edition. Springer,

New York, U.S.A.

Walser, J.-C., Sperisen, C., Soliva, M. & Scheidegger, C. (2003) Fungus-specific microsatellite

primers of lichens: application for the assessment of genetic variation on different spatial

scales in *Lobaria pulmonaria*. *Fungal Genetics and Biology* **40**: 72–82.

Wareborn, I. (1979) Reproduction of two species of land snails in relation to calcium salts in the

foerna layer. *Malacologia* **18:** 177–180.

Wickham, H. (2016) ggplot2: Elegant Graphics for Data Analysis. Springer-Verleg, New York,

U.S.A.

3. Forage Choice and Preference Affect Lichen Biomass Consumed by Native and Introduced Snail Species

3.1 Abstract

Overgrazing by gastropods is a concern for several lichen species, including *Lobaria pulmonaria*. Earlier research has examined gastropod-lichen interactions and how lichenivory is affected by lichen palatability, lichen frequency, and environmental factors such as tree type. However, it is not understood how lichen palatability and grazing pressure varies in relation to lichen species diversity and forage choice. In this study we measured the amount of lichen biomass consumed with varying lichen forage choice by two species of lichenivorous snails. We hypothesized that lichen biomass consumption would increase with greater forage choice for a native (*Cepaea hortensis*) and an introduced snail (*Cepaea nemoralis*). We compared their lichen consumption in a cafeteria experiment in controlled conditions. Total replicates for the feeding experiment using both snail species was 150. As predicted, snails consumed significantly more lichen biomass when lichen forage choice increased; however, snails still consumed some biomass of all lichen species in all forage choice treatments. The introduced snail species did not consume significantly more biomass compared to the native one. Distinct lichen preferences for *Parmelia squarrosa* and *Platismatia glauca* emerged based on the available forage choice while the biomass of *L. pulmonaria* consumed significantly decreased with each addition of alternative lichen species. Notably in the presence of common lichen species *Platismatia glauca* and *Pamelia squarrosa, L. pulmonaria* experienced less lichenivory indicating that lichen forage choice may be an important determinant of *L. pulmonaria* lichenivory. Therefore, diverse foliose lichen communities may be more resilient to gastropod grazing.

3.2 Introduction

Globally, many lichen species are in decline due to air pollution (e.g., van Herk 2001), acid rain (Gauslaa 1995; Gilbert 1986), habitat loss, and lichenivory. Gastropod lichenivory is a major concern for several lichen species, and recent studies have identified moderate (Clyne *et al.* 2019), severe, and extreme (Chapter 2) lichenivory within northeastern North America. Gastropod lichenivory constrains early development and growth of the cephalolichen *Lobaria pulmonaria* (Asplund & Gauslaa 2008) and has led to dramatic declines in populations of the cyanolichen *Pseudocyphellaria citrina* (Gyeln.) Lücking, Moncada & S. Stenroos in forests of Norway (Gauslaa 2008). In Canada, the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) has identified gastropod lichenivory as a threat for the endangered lichen species *Erioderma pedicellatum* (Hue) P.M. Jørg. in Atlantic Canada (Environment and Climate Change Canada 2018). Considerable research has been conducted on examining the gastropod-lichen interactions in western Europe (Asplund & Gauslaa 2007*b*, 2010*a, b*; Boch *et al.* 2011, 2015; Asplund 2011*b*; Asplund & Wardle 2017; Gauslaa *et al.* 2020) and how lichenivory is affected by lichen palatability (Gauslaa 2005; Asplund 2011*b*; Asplund & Wardle 2013; Boch *et al.* 2015), lichen frequency (Boch *et al.* 2014), and environmental factors such as tree type (Asplund *et al.* 2018). However, is it is not understood how lichen palatability and lichenivory pressure varies in relation to lichen forage choice and species diversity.

Lichen palatability is affected by a variety of factors as reviewed by Asplund (2010) but generally can be described as the capability of the lichenivore to use the nutrient and energy sources of the lichen while avoiding harm from lichen defense mechanisms (e.g., poor nutrient composition, surface toughness, toxic secondary metabolites) (Gauslaa 2005; Gadea *et al.* 2019). There is a large body of literature that provides evidence that certain lichen secondary

metabolites (LSM) act as a chemical defense and deter lichenivores (Reutimann & Scheidegger 1987; Gauslaa 2005; Pöykkö *et al.* 2005; Asplund & Gauslaa 2010*b*; Asplund *et al.* 2010*b*). Palatability varies between lichen species depending on the lichen species composition leading to the development of lichen preferences. Many gastropod species have adapted to deal with lichen defense mechanisms such as LSM (Hesbacher *et al.* 1995) and surface toughness. For example, *Lehmannia marginata* (O.F. Müller) has a specialized radula with many sharp teeth that aid in the consumption of lichen with tough surfaces (Chichester & Getz 1973).

In this study, we investigate the relationship between forage choice and palatability. Due to observed selective grazing of lichens by gastropods (Chapter 2) we hypothesize that available forage choice influences the amount of lichen grazing by two species of snails, native *Cepaea hortensis* O. F. Müller, (Pearce *et al.* 2010) and introduced *Cepaea nemoralis* Linnaeus (Grimm *et al.* 2010). If forage choice influences gastropod grazing on lichen thalli, then we predict that gastropods consume more lichen biomass when more forage choice is available.

3.3 Methods

Experimental design. We used feeding trial experiments to evaluate lichen consumption for two tree climbing snail species, *C. hortensis* and *C. nemoralis. C. hortensis* is a native species to the island of Newfoundland while *C. nemoralis* is an introduced species (John Maunder, personal communication, September 1, 2020). *C. hortensis* is known to graze on *L. pulmonaria* thalli in Norway (Gauslaa *et al.* 2006) and both snail species are observed grazing lichens in Newfoundland (Andre Arsenault, personal communication, Sept 1, 2019). Both species of snails were opportunistically harvested from the bole and branches of a backyard cherry tree (*Prunus*) in Corner Brook, NL.

We included lichen species *L. pulmonaria, Platismatia glauca*, and *Parmelia squarrosa* in cafeteria style feeding trials. Lichen species selected for the feeding trial belong to an assemblage of common lichens found in mixed-wood boreal forests in western Newfoundland. We have observed that these species had different frequency, and grazing damage in the field (Chapter 2). The major LSMs found in these species have been previously reported (Hinds & Hinds 2007) (**Table 3.1**).

Table 3.1. Studied lichen species and their major secondary compounds (from Hinds & Hinds 2007).

Species	Family	Secondary compounds
Lobaria pulmonaria[1] (L.) Hoffm.	Lobariaceae	Stictic, norstictic acid
Platismatia glauca[2] (L.) W.L.Culb. & C.F.Culb.	Parmeliaceae	Atranorin, caperatic acid
Parmelia squarrosa[2] Hale	Parmeliaceae	Atranorin, salazinic acid

[1] cephalolichen; [2] chlorolichen

Lobaria pulmonaria was collected from branches of survey trees in the Blue Gulch Watershed of Pasadena, NL and both *Platismatia glauca* and *Parmelia squarrosa* were collected from trees on the Corner Brook Stream Trail, NL. Collection was limited to one or two trees within a small area to reduce the risk of variability in LSM within species. Two chemical races of *L. pulmonaria* were identified by (Asplund 2011*a*) who found that LSM content can vary between regions.

Total replicates for the feeding experiment using both snail species was 150, including 60 replicates (20 per treatment) using *C. nemoralis* and 90 (30 per lichen forage treatment) using *C. hortensis*. Three feeding treatments included: 1) *L. pulmonaria* only 2) *L. pulmonaria* and

Platismatia glauca in combination and, 3) *L. pulmonaria*, *Platismatia glauca* and *Parmelia squarrosa* in combination. For all feeding treatments, the dry weight of total lichen biomass per individual feeding trial was 1.5 mg. Total lichen biomass was split equally between lichen species included in the feeding trail (e.g., for treatment 3) 0.5 mg of each lichen was used). Lichen material was weighed by species and photographed before the feeding experiment. Water saturated lichen thallus was placed in opaque containers with one snail specimen for 24 hours (**Fig 3.1**). The snails were fasted for 24 hours prior to the experiment and were fed a diet which included all three lichen species prior to fasting to eliminate the possibility of bias towards certain lichen species. After 24 hours of air-drying lichen biomass was re-weighed.

Figure 3.1. Experimental set-up for cafeteria style feeding experiment using two lichen species, *L. pulmonaria* and *Platismatia glauca*. Lichen material and snail individuals were placed in 10 cm diameter opaque white containers and were covered with white cloth coverings for the duration of the experiment.

   Statistical Analysis*.* The stats package of R 4.0.0 (R Core Team 2019) was used to create models for all analyses. A generalized linear model (with α threshold = 0.05) was used to test whether lichen biomass consumed differed depending on lichen treatment and snail type. The

model form used was Lbc = β0+ βLt + βSt + residual error, where the response variable Lbc =

dry lichen mass change, and the explanatory variable Lfc = lichen forage choice and, St = snail

type. The gamma distribution with an identity link was used to fit the data in the model. The

residual values were checked for assumptions of normality, independence, and homogeneity

using the DHARMa package (Hartig 2021). The KS test indicated that the deviance was slightly

non-normal, but the test was deemed sound given the highly significant trends. We used a

Pearson's chi-squared hypothesis test to find whether forage choice significantly influenced

lichen biomass consumed. Pearson correlations were used to test the relationship among alternate

forage treatments. The package ggplot2 (Wickham 2016) in R studio (R Core Team 2019) was

used to create all figures.

3.4 Results

We found that lichen forage choice was a significant determinant of the total amount of

lichen biomass consumed (χ^2 **= 38.60; df = 2; p < 0.01**). Snails consumed the least lichen

biomass when only *L. pulmonaria* was provided (**β = 0.16; SE = 0.12; p = 0.17**), and

significantly more biomass when *P. glauca* (**β = 1.12; SE = 0.16; p < 0.00**) and *Platismatia*

glauca and *Parmelia squarrosa* were additionally provided (**β = 1.31; SE = 0.16; p < 0.00**).

When we considered the individual species biomass consumption, *L. pulmonaria*

consumption significantly decreased with the addition of *P. glauca* (**β = -0.71; SE = 0.20; p <**

0.00) and when *Platismatia. glauca* and *Parmelia squarrosa* were included (**β = -0.53; SE =**

0.21; p = 0.01; Fig. 3.2). When we included all three lichen species, snails consumed

significantly more *Platismatia glauca* (**β = 1.49; SE = 0.34; p < 0.00**, and *Parmelia squarrosa,*

(**β = 7.56; SE = 0.34; p < 0.00**). Overall, in presence of other lichen species, *L. pulmonaria*

consumption significantly decreased (**Fig. 3.4**), but overall lichen biomass consumed

significantly increased (**Fig 3.2**).

We found no significant difference in terms of lichen biomass consumed per 24 hour

feeding trial between native snail species *C. nemoralis* and introduced *C. hortensis* **(β = -0.20;**

SE = 0.28; p = 0.49). When we considered just *L. pulmonaria* biomass consumption, we found

no significant difference between snail species **(β = -0.07; SE = 0.14; p = 0.62; Fig 3.5).**

Table 3.2. Average lichen biomass (DM) consumed by snails in 24 h (in mg $\pm$ Standard Error)

when one, two and three lichen species were provided as forage.

	Average lichen biomass (mg) consumed per forage choice		
	1 Species	**2 Species**	**3 Species**
L. pulmonaria	1.06 $\pm$ 0.13	0.37$\pm$0.05	0.53 $\pm$0.13
Platismatia glauca		**3.25** $\pm$0.38	2.02 $\pm$0.28
Parmelia squarrosa			**8.09** $\pm$0.27

Total lichen biomass before start of grazing: 15 mg
Bold font indicates the species with the highest average lichen biomass consumed
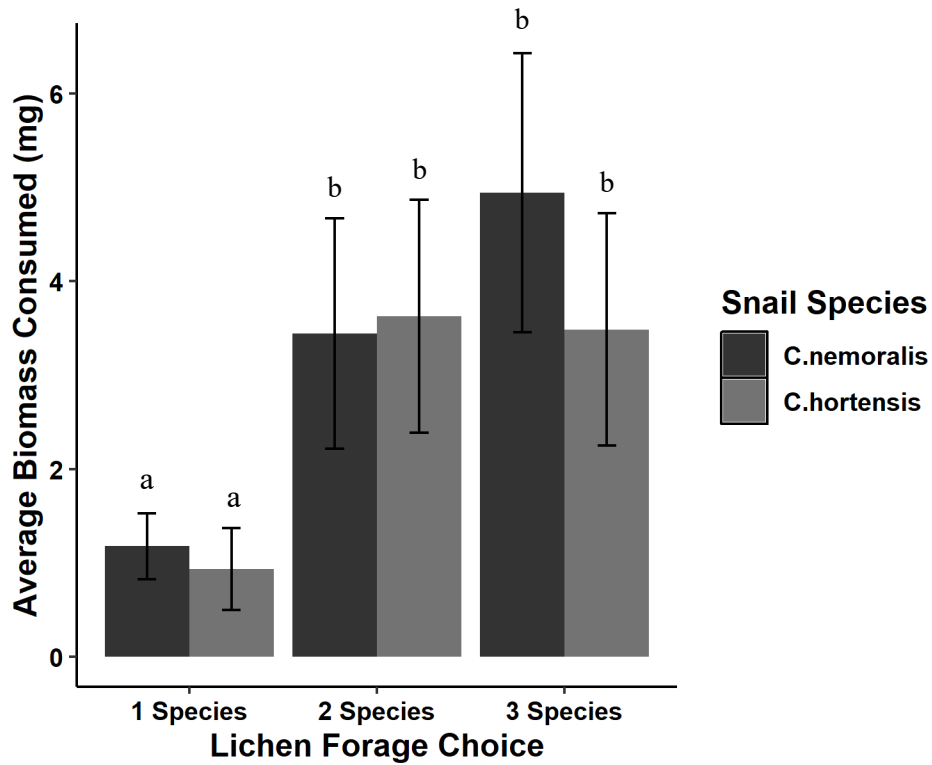
Figure 3.2. Average lichen biomass consumed ± standard error by two snail species (*C. hortensis,* and C. *nemoralis*) in mg for three forage treatments. Error bars marked with different letters are significantly different. Treatments with one species included *L. pulmonaria*, treatments with two species included *L. pulmonaria* and *Platismatia glauca*, and treatments with three species included *L. pulmonaria, Platismatia glauca* and *Parmelia squarrosa*.
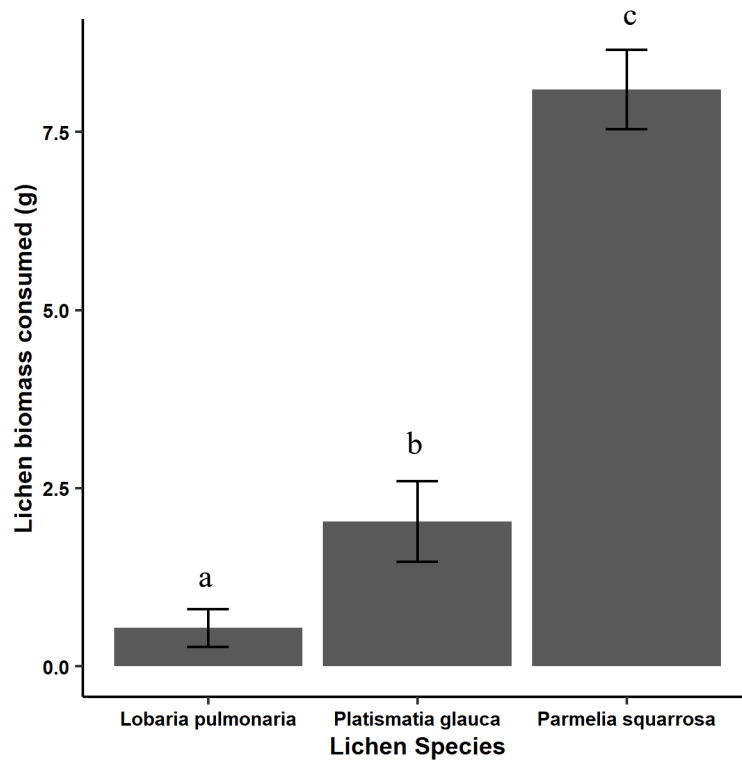
Figure 3.3. Average biomass (DM) of L. pulmonaria*, Platismatia glauca* and *Parmelia squarrosa* consumed in mg ± standard error by snails (*C. hortensis*, and *C. nemoralis*) in lichen feeding experiment treatment 3. Error bars marked with different letters are significantly different
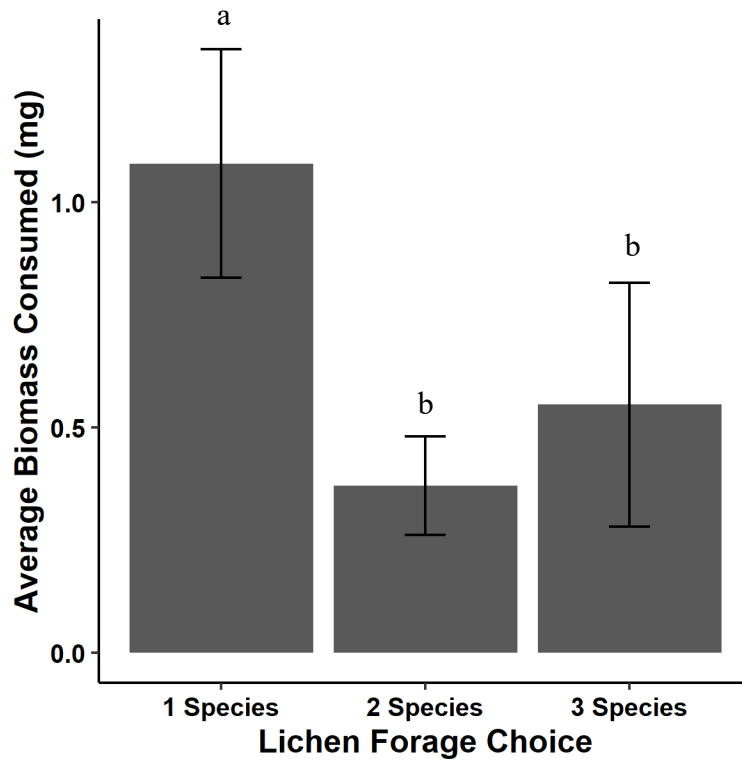
Figure 3.4. Average biomass (DM) of *L. pulmonaria* consumed by snail species (*C. hortensis*, and *C. nemoralis*) in mg ± standard error for forage choice treatments. Error bars marked with different letters are significantly different. Treatments with one species included *L. pulmonaria*, two species included *L. pulmonaria* and *Platismatia glauca*, and three species included *L. pulmonaria*, *Platismatia glauca* and *Parmelia squarrosa*.

Figure 3.5. Average biomass consumed in mg ± standard error per feeding experiment by two snail species, native *C. nemoralis* and introduced *C. hortensis* for all three forage choice treatments. Error bars marked with different letters are significantly different.

3.5 Discussion

Gastropod lichenivory can dramatically alter lichen communities and restrict lichen development and growth (Asplund & Gauslaa 2007*b*; Gauslaa 2008; Asplund *et al.* 2010*a*). Palatability is an important variable in herbivory regimes (Rundel 1978); however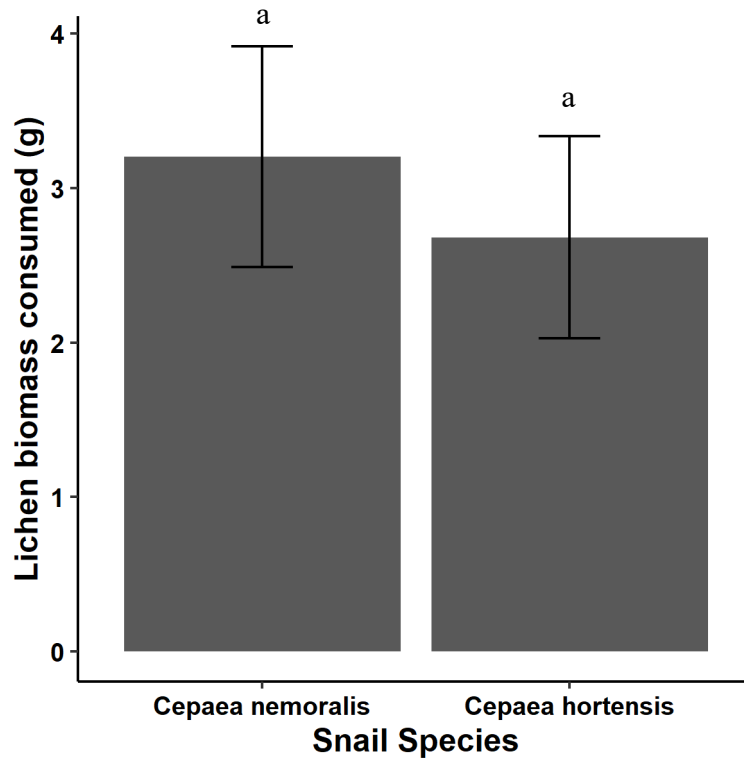, it is not understood how lichen palatability and lichenivory pressure varies in relation to lichen forage choice. In Chapter 2 we found that lichenivory was severe and widespread within our western Newfoundland study area and was especially significant for the cephalolichen *L. pulmonaria*. Understanding of how the forage choice affects gastropod lichenivory will provide useful information for the mitigation of the threat of gastropod lichenivory to foliose tree-lichens.

Lichen forage choice may enable mitigation of lichenivory defenses by snails

In this study we evaluated how varying forage choice affects lichen biomass consumed by native and introduced tree-climbing snail species. Overall, snails consumed all lichen species to some extent for all feeding experiments, although snails consumed significantly more lichen biomass with increased forage choice (**Fig 3.2**). When we provided snails with all three lichen species, they consumed significantly more of the chlorolichens *Platismatia glauca* and *Parmelia squarrosa* in comparison to the cyphalolichen *L. pulmonaria*. Notably, we found that the amount of *L. pulmonaria* consumed significantly decreased with increased forage choice (**Fig 3.4**). If we had tested the preference of each lichen species separately before integrating the effect of forage choice and species diversity, we would see the effect of forage choice more clearly. The study area where *L. pulmonaria* for the feeding experiments was collected and the area where we evaluated lichenivory for *L. pulmonaria* as severe to extreme had low lichen species diversity (**Chapter 2 Table 2.3**). It is possible that *L. pulmonaria* is more resistant to lichenivory in the

presence of more diverse foliose tree-lichen communities because it is a less palatable lichen species compared to the two chlorolichens. To confirm this, it would be necessary to include LSM measurements to understand the drivers of lichen palatability more intimately.

Palatability for gastropods is dependent on the nutritional value of the lichens and any defensive mechanisms they have developed (Rundel 1978). Many lichens are protected by chemical defenses that deter lichenivores (Lawrey 1983*a*; Gauslaa 2005; Asplund 2011*b*). Chemical defenses vary between and within species in secondary metabolite composition and concentration. Although we did not quantify LSM content, *L. pulmonaria* in Norway is known to be defended by LSM which deter gastropod lichenivory (Asplund *et al.* 2009). In a study which compared gastropod lichenivory of one cephalolichen and one cyanolichen, Gauslaa (2008) found that *L. pulmonaria* was avoided in the presence of less chemically protected *Pseudocyphellaria citrina*. The method by which plant-feeding gastropods can identify and avoid overconsumption of plant secondary metabolites was described by Speiser (2001), gastropods use food sampling where they consume a small amount of the plant at first to learn whether to accept or reject it as a good food source; and the total mass consumed is dependent on the nutritional quality, plant secondary metabolite content, palatability, and availability (Speiser 2001).

Snails showed distinct lichen preference

The three lichen species used in our feeding experiment appeared to have different levels of palatability resulting in distinct preferences (**Fig 3.3**). Additionally, lichen preference changed based on the available forage choice (**Table 3.2**). When we provided snails with all three lichen species, they consumed significantly more *Platismatia glauca* and *Parmelia squarrosa,* and *L. pulmonaria* consumption significantly decreased with increasing choice in forage (**Fig 3.4**).

L. pulmonaria is a cephalolichen, formed from a fungal species, cyanobacteria and green algae (Nash 2008)*.* The cyanobacteria in *L. pulmonaria* fix nitrogen. Both *Platismatia glauca* and *Parmelia squarrosa* are chlorolichens containing green algal symbionts and contain much less nitrogen content. This makes *L. pulmonaria* a rich nitrogen source for lichenivores. However, *L. pulmonaria* contains medullary LSM including the stictic acid complex, and a high concentration of these depsidones that deter gastropod lichenivory (Asplund & Gauslaa 2007*a*; Asplund *et al.* 2009). *Parmelia squarrosa* contains atranorin and salazinic acid (Smith *et al.* 2009). Černajová and Svoboda (2014) found that LSM concentration in a lichen of the same genus, *Parmelia sulcata* Taylor, also including salazinic acid, deterred gastropods. *Platismatia glauca* contains atranorin and caperatic acid. Lawrey (1983) found that caperatic acid deterred lichenivores in epiphytic foliose lichen *Usnocetraria oakesiana* (Tuck.) M.J. Lai & J.C. Wei. In this study, the chemical defense of *L. pulmonaria* may have been a strong enough concentration to deter lichenivory in the presence of alternate forage, despite the appeal of its rich nitrogen content. *Parmelia squarrosa* and *Platismatia glauca* are very common, however; they may have had a weaker concentration of chemical defense making them preferred based on their overall palatability. Lichen preference by gastropods is a result of the overall lichen palatability (Gerson & Seaward 1977; Rundel 1978) as well as the environment where the lichen is found and any adaptations the gastropod may have developed (Lawrey 1983*a*; Baur *et al.* 1994; Hesbacher *et al.* 1995; Gadea *et al.* 2019). Palatability is based on the capability of the lichenivore to use the nutrient and energy content of the lichen while avoiding harm resulting from lichen defense mechanisms (Gauslaa 2005; Gadea *et al.* 2019). To reduce palatability, lichens have evolved different adaptations including low nutritional value and strong chemical defense (Gauslaa 2005; Černajová *et al.* 2014; Wieners *et al.* 2018), as well as surface toughness (Asplund 2010; Lucas

et al. 2000). However, gastropods have developed the ability to increase palatability, for instance by adapting to LSM (Hesbacher *et al.* 1995) or developing specialized mouthparts to consume tough lichen cortices (Chichester & Getz 1973). It is possible that the functional traits including surface texture, surface toughness and thallus thickness could have also influenced lichen palatability by snails in our study. Consumption of lichen with more textured thalli such as *L. pulmonaria* may be more energy intensive for snails and therefore be avoided. In this study, *L. pulmonaria* was the least palatable lichen species, *Platismatia glauca* is more palatable, and *Parmelia squarrosa* was the most palatable and the preferred species when all three species were available (**Table 3.2**). Snails appear to prefer *Platismatia glauca* and *Parmelia squarrosa* to *L. pulmonaria*, so we speculate that the latter species has higher LSM content or is less nutritionally valuable than the former two. If we had tested the preference of each lichen species separately and measured LSM content before integrating the effect of forage choice and species diversity this relationship would be clearer.

Possible health benefits of lichen secondary metabolites for gastropods

Both preferred lichen species contain atranorin. Atranorin is known to absorb light in lichens that prefer low light intensities (Rao & LeBlanc 1965. Villalba and Provenza (2007) found that in plant communities where species diversity was higher, herbivores selectively consume a variety of plants to cope with, and benefit from, plant secondary metabolites (PSM). Researchers posit that at low concentrations some PSM provide treatment for health imbalances in herbivores and are vital to the health of both plants and herbivores (Forbey *et al.* 2009). In a meta-analysis that investigated the effect of plant defenses on herbivores for common and rare plants, Smilanich *et al.* (2016) identified a widespread pattern among plants and herbivores of

the beneficial effects of PSM on herbivore performance (i.e., herbivore development time, feeding data, growth rates, and survivorship). Research has shown that PSM aid herbivores in controlling internal parasites, restricting the overgrowth of internal fungal and bacterial populations, enhancing nutrition (Villalba & Provenza 2007), enhancing reproduction, moderating thermoregulation, avoiding predation, and increasing alertness (Forbey *et al.* 2009). It is possible that atranorin provides some form of medicinal benefit to snail species included in this feeding experiment, although the relationship is speculative.

Lichenivory did not vary between snail species

In this study, we used two species of snails in feeding experiments, *C. hortensis* is a native snail to the region and *C. nemoralis* an invasive snail, both species are known to consume arboreal lichen as part of their generalist diet (Gauslaa *et al.* 2006). There was no significant difference in lichen consumption between the two species of snail (**Fig 3.5**). Lichen palatability varies between snail species in line with their unique abilities to deal with lichen defense mechanisms or different adaptations to use energy and nutrients of the lichen (Asplund 2010). In fact, snail species inhabiting the same ecosystem may even prefer different lichen species (Baur *et al.* 1992, 1994; Fröberg *et al.* 1993). The two snail species we used were of the same genera but differed in their native distribution. The findings indicate that the palatability of the lichens included in the experiment was similar for both snail species and/or both snail species used similar processes to avoid lichen defense mechanisms. The introduced *C. nemoralis* does not represent a more aggressive lichenivore than the native *C. hortensis* under these experimental conditions.

3.6 Conclusion and Future Recommendations

This study measured the amount of lichen biomass consumed with varying forage choice by two species of lichenivorous snails, native *C. hortensis* and introduced *C. nemoralis*. Overall, snails consumed significantly more lichen biomass when forage choice increased (**Fig 3.2**). Snails showed distinct lichen preferences and tolerances; snail preference changed based on the amount of forage choice where *Platismatia glauca* and *Parmelia squarrosa* were significantly more consumed compared to *L. pulmonaria* when all lichen were available (**Table 3.2**). *Lobaria pulmonaria* consumption significantly decreased with each addition of alternative lichen species (**Fig 3.4**), and therefore, *L. pulmonaria* may be more resilient to overgrazing in forested environments with higher lichen species diversity. We found no significant difference in lichen consumption between the two species of snail (**Fig 3.5**), the introduced *C. nemoralis* does not represent a more aggressive lichenivore compared to *C. hortensis*.

We recommend the integration of LSM measurements in future research on this topic to understand the drivers of lichen palatability more intimately as well as an experimental design that tests the preference of each lichen species separately before integrating the effect of forage choice and species diversity.

3.7 References

Asplund, J. (2010) Lichen-gastropod interactions – Chemical defence and ecological consequences of lichenivory". PhD Thesis: Norwegian University of Life Sciences, Ås, Norway.

Asplund, J. (2011*a*) Chemical races of *Lobaria pulmonaria* differ in palatability to gastropods. *The Lichenologist* **43**: 491–494.

Asplund, J. (2011*b*) Snails avoid the medulla of *Lobaria pulmonaria* and *L. scrobiculata* due to presence of secondary compounds. *Fungal Ecology* **4**: 356–358.

Asplund, J. & Gauslaa, Y. (2007*a*) Content of secondary compounds depends on thallus size in the foliose lichen *Lobaria pulmonaria*. *The Lichenologist* **39**: 273–278.

Asplund, J. & Gauslaa, Y. (2007*b*) Mollusc grazing limits growth and early development of the old forest lichen *Lobaria pulmonaria* in broadleaved deciduous forests. *Oecologia* **155**: 93–99.

Asplund, J. & Gauslaa, Y. (2010*b*) The gastropod *Arion fuscus* prefers cyanobacterial to green algal parts of the tripartite lichen *Nephroma arcticum* due to low chemical defence. *The Lichenologist* **42**: 113–117.

Asplund, J. & Wardle, D. A. (2013) The impact of secondary compounds and functional characteristics on lichen palatability and decomposition. *Journal of Ecology* **101**: 689–700.

Asplund, J. & Wardle, D. A. (2017) How lichens impact on terrestrial community and ecosystem properties. *Biological Reviews* **92**: 1720–1738.

Asplund, J., Solhaug, K.A. & Gauslaa, Y. (2009) Fungal depsidones-an inducible or constitutive defence against herbivores in the lichen *Lobaria pulmonaria*? *Basic and Applied Ecology* **10**: 273-287.

Asplund, J., Larsson, P., Vatne, S. & Gauslaa, Y. (2010*a*) Gastropod grazing shapes the vertical distribution of epiphytic lichens in forest canopies. *Journal of Ecology* **98**: 218–225.

Asplund, J., Solhaug, K. A. & Gauslaa, Y. (2010*b*) Optimal defense: Snails avoid reproductive parts of the lichen *Lobaria scrobiculata* due to internal defense allocation. *Ecology* **91**: 3100–3105.

Asplund, J., Strandin, O. V. & Gauslaa, Y. (2018) Gastropod grazing of epiphytic lichen-dominated communities depends on tree species. *Basic and Applied Ecology* **32**: 96–102.

Baur, A., Baur, B. & Fröberg, L. (1992) The effect of lichen diet on growth rate in the rock-dwelling land snails *Chondrina clients* (Westerlund) and *Balea perversa* (Linnaeus). *Journal of Molluscan Studies* **58**: 345–347.

Baur, A., Baur, B. & Fröberg, L. (1994) Herbivory on calcicolous lichens: Different food preferences and growth rates in two co-existing land snails. *Oecologia* **4**: 313-319.

Boch, S., Prati, D., Werth, S., Rü Etschi, J. & Fischer, M. (2011) Lichen endozoochory by snails. *PLoS ONE* **6**.

Boch, S., Fischer, M. & Prati, D. (2014) To eat or not to eat - Relationship of lichen herbivory by snails with secondary compounds and field frequency of lichens. *Journal of Plant Ecology* **8**: 642–650.

Cameron, R. (2009) Are introduced gastropods a threat to endangered lichens? *Canadian Field-Naturalist* **123**: 169–171.

Campbell, J. & Fredeen, A. L. (2004) *Lobaria pulmonaria* abundance as an indicator of macrolichen diversity in Interior Cedar-Hemlock forests of east-central British Columbia. *Canadian Journal of Botany* **7***:* 970-982*.*

Černajová, I., and Svoboda, D. (2014) Lichen compounds of common epiphytic *Parmeliaceae* species deter gastropods both in laboratory and in Central European temperate forests. *Fungal Ecology* **11**: 8-16.

Chichester, L. & Getz, L. (1973) The terrestrial slugs of northeastern North America. *Sterkiana* **51**: 11–41.

Clyne, A. B., Cleavitt, N. L. & Fahey, T. J. (2019) Terrestrial gastropod grazing on macrolichens in a northern broadleaf–conifer forest. *Northeastern Naturalist* **26**: 261.

Environment and Climate Change Canada (2018) *Recovery Strategy for the Boreal Felt Lichen (Erioderma pedicellatum), Atlantic Population, in Canada*. Ottawa, Canada.

Forbey, J. S., Harvey, A. L., Huffman, M. A., Provenza, F. D., Sullivan, R. & Tasdemir, D. (2009) Exploitation of secondary metabolites by animals: A response to homeostatic challenges. *Integrative and Comparative Biology* **49**: 314–328.

Fröberg, L., Baur, A. & Baur, B. (1993) Differential herbivore damage to calcicolous lichens by snails. *The Lichenologist* **25**: 83–95.

Gadea, A., Charrier, M., Fanuel, M., Clerc, P., Daugan, C., Sauvager, A., Rogniaux, H., Boustie, J., le Lamer, A. C. & Lohézic - Le Devehat, F. (2019) Overcoming deterrent metabolites by gaining essential nutrients: A lichen/snail case study. *Phytochemistry* **164**: 86–93.

Gauslaa, Y. (1995) The Lobarion, an epiphytic community of ancient forests threatened by acid rain. *The Lichenologist* **27**: 59–76.

Gauslaa, Y. (2005) Lichen palatability depends on investments in herbivore defence. *Oecologia* **143**: 94–105.

Gauslaa, Y. (2008) Mollusc grazing may constrain the ecological niche of the old forest lichen *Pseudocyphellaria crocata*. *Plant Biology* **10**: 711–717.

Gauslaa, Y., Holien, H., Ohlson, M. & Solhøy, T. (2006) Does snail grazing affect growth of the old forest lichen *Lobaria pulmonaria*? *The Lichenologist* **38**: 587–593.

Gauslaa, Y., Larsson, P. & Asplund, J. (2020) Selective feeding by gastropods in *Lobaria scrobiculata* allows quantification of intrathalline anatomical layers. *The Lichenologist* **42**(5): 621-625.

Gerson, U. & Seaward, M. (1977) Lichen-Arthropod Associations. *The Lichenologist* **5**: 434–443.

Gilbert, O. L. (1986) Field evidence for an acid rain effect on lichens. *Environmental Pollution. Series A, Ecological and Biological* **40**: 227–231.

Grimm, W. F., Forsyth, R. G., Schueler, F. W. & Karstad, A. (2010) *Identifying land snails and slugs in Canada: introduced species and native genera*. Canadian Food Inspection Agency.

Hartig, F. (2021) DHARMa: Residual Diagnostics for Hierarchical (Multi-level/Mixed) Regression Models. R package version 0.4.1. http://CRAN.R-project.org/package=DHARMa.

Hesbacher, S., Baur, B., Baur, A. & Proksch, P. (1995) Sequestration of lichen compounds by three species of terrestrial snails. *Journal of Chemical Ecology* **2**(21): 233-246.

Hinds, J. & Hinds, P. (2007) *The macrolichens of New England: descriptions keys, conservation status*. New York Botanical Garden Press, New York, U.S.A.

Lawrey, J. D. (1983*a*) Lichen herbivore preference: a test of two hypotheses. *American Journal of Botany* **8**(70): 1188-1194.

Lawrey, J. D. (1983b) Vulpinic and pinastric acids as lichen antiherbivore compounds: contrary evidence. *The Bryologist* **4**(86): 365-369.

Lucas, P., Turner, I., Dominy, N., Yamashita, N. (2000) Mechanical defences to herbivory. *Annals of Botany* **5**: 913-920.

Melo, M. G. D., dos Santos, J. P. A., Serafini, M. R., Caregnato, F. F., de Bittencourt Pasquali, M. A., Rabelo, T. K., da Rocha, R. F., Quintans, L., de Souza Araújo, A. A., da Silva, F. A., Moreira, J. C. F. & Gelain, D. P. (2011) Redox properties and cytoprotective actions of atranorin, a lichen secondary metabolite. *Toxicology in Vitro* **25**: 462–468.

Nash III, T. H. (2008) *Lichen Biology, second edition*. Cambridge University Press.

Pearce, T. A., Olori, J. C. & Kemezis, K. W. (2010) Land snails from St. Elzear Cave, Gaspé Peninsula, Quebec: Antiquity of *Cepaea hortensis* in North America. *Annals of Carnegie Museum* **79**: 65–78.

Pöykkö, H., Hyvärinen, M. & Bačkor, M. (2005) Removal of lichen secondary metabolites affects food choice and survival of lichenivorous moth larvae. *Ecology* **10**(86): 2623-2632.

R Core Team. 2019. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.r-project.org/.

Rao, D. N. & LeBlanc, F. (1965) A possible role of atranorin in the lichen thallus. *The Bryologist* **3**: 284-288.

Reutimann, P. & Scheidegger, C. (1987) Importance of lichen secondary products in food choice of two oribatid mites (Acari) in an alpine meadow ecosystem. *Journal of Chemical Ecology* **13**: 363–369.

Rundel, P. W. (1978) The ecological role of secondary lichen substances. *Biochemical Systematics and Ecology* **3**: 157-170.

Smilanich, A. M., Fincher, R. M. & Dyer, L. A. (2016) Does plant apparency matter? Thirty years of data provide limited support but reveal clear patterns of the effects of plant chemistry on herbivores. *New Phytologist* **210**: 1044–1057.

Smith, C., Aptroot, A., Coppins, B., Fletcher, A., Gilbert, O., James, P. & Wolseley, P. (Eds.) (2009) *The Lichens of Great Britain and Ireland.* British Lichen Society.

Speiser, B. (2001) Food and feeding behaviour. *The Biology of Terrestrial Molluscs* (G. Barker, ed): pp. 259–259. Wallingford, England.

Vatne, S., Solhøy, T., Asplund, J. & Gauslaa, Y. (2010*a*) Grazing damage in the old forest lichen *Lobaria pulmonaria* increases with gastropod abundance in deciduous forests. *The Lichenologist* **5**: 615–619.

Villalba, J. & Provenza, F. D. (2007) Self-medication and homeostatic behaviour in herbivores: learning about the benefits of nature's pharmacy. *Animal* **1**: 1360-1370.

Wickham, H. (2016) ggplot2: Elegant graphics for data analysis. *Springer-Verlag.* New York. U.S.A. https://cran.r-project.org/web/packages/ggplot2/index.html

Wieners, P. C., Bilger, W. & Gauslaa, Y. (2018) Carbon-based secondary compounds in the lichen *Hypogymnia physodes* deter detrivorous woodlice. *Fungal Ecology* **31**: 54–58.

4. Summary

Epiphytic lichens are sensitive to many environmental stressors such as air pollution, habitat disturbance, climate change (Nascimbene *et al.* 2016) and increasingly, gastropod lichenivory (Asplund & Gauslaa 2007; Environment and Climate Change Canada 2018). Within northeastern Canada, climate change is projected to lead to increased annual rainfall and warmer winters (Environment and Climate Change Canada 2016) that will presumably increase gastropod populations (Sternberg 2000; Willis *et al.* 2006). In addition, climate change accelerates the introduction and spread of many invasive species while reducing the resilience of ecosystems to biological invasion (Masters & Norgrove 2010). It is therefore important to monitor lichen populations to identify any threats to their survival. This study establishes a baseline of information on gastropod lichenivory for a mixed-wood boreal forest study area in western Newfoundland and fills existing knowledge gaps in the understanding of factors affecting lichenivory. Establishing a baseline of information that can inform future research is a critical step in mitigating any threat posed to lichens by gastropod lichenivory (Nichols & Williams 2006; Allen *et al.* 2019).

In Chapter 2, I evaluated lichenivory and surveyed lichen diversity and the tree-climbing gastropod community. Gastropod lichenivory was severe to extreme and, however; different lichen species experienced very different grazing pressure within the study area indicating that lichenivory may be species specific and must be evaluated as such. *L. pulmonaria* was most severely impacted with an average of severe to extreme levels of lichenivory observed. Patterns of lichenivory were shaped by variables at the tree level, specifically, the location of lichen thalli on the tree and the type of tree (conifer or broadleaf) was an important environmental factor driving lichenivory extent for the overall lichen community. I used two methods of gastropod

collection including timed searches and arboreal pitfall traps to evaluate the tree climbing gastropod community. I recommend the use of these two methods in combination as well as the addition of more frequent searches across a longer time frame to fully capture the seasonal flux within the gastropod community. Slugs were the most common group within the tree climbing terrestrial gastropod community and *Lehmannia marginate* and *Arion hortensis* were the most common tree-climbing slug species. This is the first study to describe the current state of gastropod lichenivory in mixed forests on the island of Newfoundland and will function as a source of baseline data for further research and monitoring.

In Chapter 3, I quantified the amount of lichen biomass consumed with varying lichen species diversity by two species of lichenivorous snails. I used a cephalolichen, *L. pulmonaria,* identified as experiencing severe to extreme lichenivory in Chapter 2, as well as two common boreal chlorolichen species, *P. glauca* and *P. squarrosa*. Snail species included native *C. hortensis* and introduced *C. nemoralis*. Snails consumed significantly more lichen biomass when forage choice increased. However, distinct lichen preferences emerged where *P. glauca* and *P. squarrosa* were consumed significantly more, and *L. pulmonaria* was consumed significantly less, when all three lichen species were available. Therefore, *L. pulmonaria* may be more vulnerable to lichenivory in forests with low species diversity, and more resilient to lichenivory in forests with greater species diversity such as older and less disturbed forests. There was no significant difference in lichen consumption between the two species of snail and therefore, introduced *C. nemoralis* is not a more aggressive lichenivore.

4.1 Limitations and Opportunities

As a result of COVID-19 restrictions, experimental design for my thesis changed frequently and required a high degree of adaptability and problem solving. I faced many challenges but ultimately, I believe that these obstacles strengthened the thesis. For example, the lab-based feeding experiments in Chapter 3 were a result of uncertain fieldwork prospects but ultimately provided a very compatible addition that broadened the scope of the thesis.

Because of the short duration of this study, I did not study the seasonal, annual, and regional variation in gastropod community composition, and gastropod identifications were not completed. Identification of the gastropod species responsible for lichenivory would be a considerable contribution to the current literature and could be a topic of focus for future research.

Quantification of lichen secondary metabolites for Chapter 3 was outside of the scope of this thesis; however, without knowing the chemical content of the lichens it was impossible to say lichen consumption occurred because of chemical defense or other defense mechanisms. Results from Chapter 3 highlighted the confounding effects of species diversity and species preference. If feeding experiments had been carried out for each lichen species individually before introducing different levels of species diversity it would have been possible to identify the effect of species preference before investigating the effect of species diversity.

One critique of the experimental design of Chapter 3, which most likely affected the resulting models was the omission of the individual snail effect as a factor influencing the biomass of lichen consumed. Inclusion of the individual effect of snails would have controlled for variance between snail individuals and may have helped to better explain why certain snails

consumed more lichen. The omission of relevant explanatory variables resulted in omitted variable bias, which reduces the standard error of the model and increases risk of type I error.

4.2 Future Research

Although this study has established valuable baseline data, more research is required to understand lichenivory in Newfoundland. I recommend that future research survey a greater number of sites across Newfoundland over a longer time frame for lichenivory and tree-climbing gastropod community composition. This research will provide a clearer resolution of the extent and severity of lichenivory throughout the province. The severe to extreme level of lichenivory I found for *L. pulmonaria* within the study area merits further research to identify how lichenivory impacts survival, growth, and reproduction . To that effect it would be useful for further research to determine whether *L. pulmonaria* in Newfoundland represents a less protected chemical race compared to those in the rest of Atlantic Canada and Europe.

The following recommendations for future research may further improve the understanding of the effect of species diversity and species preference on lichen consumption: Future research should (1) include a feeding experiment which integrates lichen secondary metabolite (LSM) measurements to understand the drivers of lichen palatability more intimately, (2) the inclusion of snail biomass and snail individual effect, and (3) a modified experimental design that focuses more on testing species preference in addition to species diversity. Further research should focus on providing further validation of the grazing patterns that exist such as whether grazing was due to preference or avoidance of certain lichen species and if that preference or avoidance correlates with LSM content and concentration. Using these recommendations, it would be possible to provide better evidence to test the hypothesis that

snails may vary their lichenivory to maximize nutritional exploitation and minimize toxicity from LSM in lichens.

4.3 Contributions

My thesis makes a significant contribution to the evaluation and understanding of lichenivory in western Newfoundland and beyond. I have provided a foundational baseline of information on the current state of lichenivory in a mixed-wood forest of western Newfoundland and identified severe and extreme lichenivory of a flagship lichen species, *L. pulmonaria*, that increases with gastropod abundance. It is my hope that the results and recommendations made in my research will serve as a starting point for more research on lichenivory in Newfoundland and beyond. My findings from Chapter 3 begin to unravel how gastropod preference, species diversity and lichen palatability affect lichenivory and offer an interesting perspective linking opposing perspectives on select lichen secondary metabolites as lichenivore deterrents or attractants. My finding that *L. pulmonaria* is consumed less in the presence of greater species diversity has implications for the conservation and management of ecosystems hosting this species and provides more evidence for the idea that more diverse ecosystems have greater resistence to disturbance. As climate change continues to change ecosystems and lichen species are faced with greater disturbances it is important to establish how resiliency can be strengthened and maintained. Lichens are important members of all ecosystems where they are found and deserve to be rigorously studied and conserved.

4.4 References

Allen, J. L., McMullin, R. T., Tripp, E. A. & Lendemer, J. C. (2019) Lichen conservation in North America: a review of current practices and research in Canada and the United States. *Biodiversity and Conservation* **28**: 3103–3138.

Asplund, J. & Gauslaa, Y. (2007) Mollusc grazing limits growth and early development of the old forest lichen *Lobaria pulmonaria* in broadleaved deciduous forests. *Oecologia* **155**: 93–99.

Boch, S., Fischer, M. & Prati, D. (2015) To eat or not to eat—relationship of lichen herbivory by snails with secondary compounds and field frequency of lichens. *Journal of Plant Ecology* **8**: 642–650.

Environment and Climate Change Canada (2018) *Recovery Strategy for the Boreal Felt Lichen (Erioderma pedicellatum), Atlantic Population, in Canada*. Ottawa, ON.

Environment and Climate Change Canada (2016) *Climate data and scenarios for Canada: Synthesis of recent observation and modelling results*. Ottawa, ON.

Herk. C. M. (2001) Bark pH and susceptibility to toxic air pollutants as independent causes of changes in epiphytic lichen composition in space and time. *The Lichenologist* **33**(5): 419-442.

Masters, G. & Norgrove, L. (2010) *Knowledge for Life: Climate Change and Invasive Alien Species*. CABI Working Paper.

Nascimbene, J., Casazza, G., Benesperi, R., Catalano, I., Cataldo, D., Grillo, M., Isocrono, D., Matteucci, E., Ongaro, S., Potenza, G., Puntillo, D., Ravera, S., Zedda, L. & Giordani, P.

(2016) Climate change fosters the decline of epiphytic *Lobaria* species in Italy. *Biological Conservation* **201**: 377–384.

Nichols, J. D. & Williams, B. K. (2006) Monitoring for conservation. *Trends in Ecology and Evolution* **21**: 668–673.

Sternberg, M. (2000) Terrestrial gastropods and experimental climate change: A field study in a calcareous grassland. *Ecological Research* **15**: 73–81.

Willis, J. C., Bohan, D. A., Choi, Y. H., Conrad, K. F. & Semenov, M. A. (2006) Use of an individual-based model to forecast the effect of climate change on the dynamics, abundance, and geographical range of the pest slug *Deroceras reticulatum* in the UK. *Global Change Biology* **12**: 1643–1657.

5. Appendices

5.1 Appendix A: Working Hypothesis for AIC

Table A.1. List of working hypotheses as predictions to be used in the AIC(c) analyses included in Chapter 2.

Variable	Model	Mechanism	Prediction	References
Lichen species	Grazing class = βLichen species + error	Varying palatability (secondary metabolites) and nutrition	Different levels of grazing on different lichen species	(Clyne *et al*. 2019; Froberg *et al*. 1993)
Lichen Abundance class	Grazing class = βAbundance class + error	More grazing = less abundance	Lichens that are more abundant will show less lichenivory	(Boch *et al*. 2015)
Tree group	Grazing class = βTree group + error	Higher populations of gastropods in deciduous broadleaved forests	Broadleaved trees will show greater lichenivory	(Clyne *et al*. 2019; Gauslaa 2006)
Thalli location	Grazing class = βThalli location + binomial error	Bole more accessible	Tree boles will show greater lichenivory	(Gauslaa 2006)
Species diversity	Grazing class= βSpecies diversity + error	Greater species diversity allows for better trade-offs in secondary metabolites	Greater species diversity will show less lichenivory	(Huntly 1991; Olff & Ritchie 1998; Bauer *et al*. 1995)

5.2 Appendix B: AIC Table for Chapter 2 lichenivory analysis

Table A.2. AIC table for tree level factors influencing observed lichenivory for all lichen species surveyed.

Explanatory Parameters	logLik	AICc	ΔAIC	Weight
Thalli location + lichen species	-124.05	274.3	0.00	0.99
Global	-115.86	283.6	9.23	0.01
Tree species + lichen species	-129.23	289.5	15.19	0.00
Lichen species	-133.77	291.4	17.09	0.00
Thalli location _ species diversity	-149.37	311.3	36.98	0.00
Thalli location	-158.32	327.1	52.71	0.00
Thalli location + DBH	-157.62	327.8	53.49	0.00
DBH + species diversity	-159.62	331.8	57.49	0.00
Tree species + thalli location	-157.89	332.8	58.45	0.00
Tree species+ species diversity	-161.33	333.1	58.74	0.00
Tree species + DBH	-160.68	338.4	64.03	0.00
Tree species	-172.14	361.3	86.96	0.00
DBH	-173.45	361.7	87.34	0.00
Lichen abundance	-175.98	362.4	88.03	0.00
	-172.00	363.3	88.93	0.00

5.3 Appendix C: AIC Table for Chapter 2 lichenivory analysis

Table A.3. AIC table for tree level factors influencing observed lichenivory for *L. pulmonaria* lichen surveyed.

Explanatory Parameters	logLik	AICc	delta	weight
Thalli location + DBH + species diversity	-59.575	132.1	0	0.337
Thalli location + species diversity	-61.412	133.5	1.39	0.169
Tree type + thalli location + DBH + species diversity	-59.335	134	1.86	0.133
DBH + species diversity	-61.829	134.3	2.22	0.111
Thalli location + DBH	-62.15	135	2.86	0.081
Thalli location	-64.114	136.7	4.56	0.035
Species diversity	-64.12	136.7	4.57	0.034
Tree type + thalli location	-62.144	137.3	5.14	0.026
Tree type + thalli location	-62.144	137.3	5.14	0.026
Tree type + species diversity	-63.802	138.3	6.17	0.015
DBH	-64.983	138.4	6.29	0.014
global	-57.978	138.6	6.51	0.013
Tree type	-66.355	141.2	9.04	0.004
Tree species	-63.483	142.3	10.16	0.002
Abundance class	-64.872	145.1	12.94	0.001