# THE RESPONSE OF LICHENS TO CHANGES IN ISOTOPIC COMPOSITION AND CONCENTRATION OF ATMOSPHERIC SULPHUR: A RECIPROCAL TRANSPLANT EXPERIMENT



MÉLANIE LYNE COUSINEAU

# The response of lichens to changes in isotopic composition and concentration of atmospheric sulphur: a reciprocal transplant experiment

by

Mélanie Lyne Cousineau

A thesis submitted

to the School of Graduate Studies

in partial fulfilment of the requirements for the degree of

Master of Science in Environmental Science

Environmental Science Programme

Memorial University of Newfoundland

St. John's, Newfoundland and Labrador

December 2003

## Abstract

Industrial and technological developments of the past 100 years have resulted in significant perturbations to the global sulphur cycle. The use of biomonitors, such as lichens, for quantifying environmental changes has grown in importance over the last decades and offers promising developments for the understanding of global elemental cycles.

The present study was designed to investigate the response of lichens to changes in atmospheric sulphur conditions, as measured by changes in sulphur isotopic composition and concentration in lichen thalli. A reciprocal transplant of lichen thalli was performed between a "polluted" and "unpolluted" site, with respect to sulphur sources and concentration. Samples were collected monthly for a period of 18 months.

Multiple regression with periodic functions was used to model the observations. All lichens showed a gradual change in isotopic composition and concentration toward the values at the transplant site, but did not reach local conditions. No long-term trend was discernible for sulphur concentration measurements in lichens transplanted to the polluted site, probably due to a significant increase in sulphur concentrations at that site over the course of the experiment. Seasonal trends were identified at both sites, and at the polluted site appeared to be closely related to anthropogenic sulphur emission patterns. Some sources of seasonal variation at the unpolluted site were unidentifiable, but influences from anthropogenic sources were identified, and influences from biogenic sources suggested. The mechanics of relocation did not affect lichen responses. It is likely that distinguishing between the organic and inorganic fractions of sulphur would have helped in interpreting results.

**Acknowledgments**

I would first like to thank my supervisor, Dr. Moire Wadleigh, for providing the opportunity to work under her tutelage, for generously lending her time and expertise, and for her patience in answering my many questions. I would also like to thank Dr. David Schneider, a member of my supervisory committee, for his help with the initial planning of the experiment and the final analyses. His assistance with statistical concepts was always useful and given with remarkable efficiency, even when from several thousand miles away. For his help in dealing with the meteorological aspects of the study, I extend my appreciation to Dr. John Jacobs.

Alison Pye merits my gratitude for her substantial help with sample analysis on the mass spectrometer, and Pam King for her laboratory expertise, always generously given. Dr. Wilf Nicholls and Madonna Bishop contributed to this study by allowing the use of the MUN Botanical Garden as a sampling site.

Moral support during my two years at Memorial University of Newfoundland was provided by many people. First and foremost, thanks goes to Sébastien Héon, who was always ready to help, either by assisting in my monthly sampling excursions or simply by lending an attentive ear. His culinary talents also provided welcome gustative distractions in times of intellectual barrenness. Jennifer Bonnell, who survived the experience of graduate school before me, deserves gratitude for her understanding and for her motivational skills. Marie Julie Roux proved to be a splendid office mate and provided moral support in times of need.

Last but not least, great thanks goes to Dr. Leonard Lye, who, despite not being a member of my supervisory committee, freely offered his expertise in statistical matters in times when it was urgently needed.

This research was supported by an NSERC Post Graduate Scholarship (A), a stipend from the School of Graduate Studies and an NSERC Discovery grant to Dr. Wadleigh.

## Table of Contents



## Chapter 1: Introduction

## Chapter 2: Materials and Methods



## Chapter 3: Results

## Chapter 4: Discussion



## Chapter 5: Summary and conclusions

## List of tables

## List of figures

# Chapter 1. Introduction

## 1.1 Statement of problem

The global sulphur cycle has undergone substantial perturbations in the past century as a result of industrial and technological developments. Industrial activities are contributing significantly to the input of sulphur gases to the atmosphere. Sulphur dioxide emissions are linked directly to environmental problems such as global warming and acid precipitation, which may in turn lead to changes in rock weathering, forest growth and ocean productivity (Schlesinger, 1991). The use of biomonitors, organisms which can serve as indicators of environmental pollutants, is growing in importance and can provide valuable information on the state of our environment. Lichens are long-lived perennial organisms characterized by a symbiotic association between a fungus and an alga or cyanobacterium (Hale, 1974). Because they lack protective mechanisms to regulate the uptake of gaseous molecules (Häffner *et al.*, 2001), lichens are particularly sensitive to air pollutants (Purvis *et al.*, 2000); they have been used for more than a century as biomonitors of air quality (Nylander, 1866).

Several studies have considered the physiological effects of sulphur and its compounds on lichens, but few have examined the response of lichens to changes in the isotopic composition of atmospheric sulphur. The present study was designed to investigate an aspect of this response, as measured by changes in the thallus of the sulphur isotopic composition and concentration. Changes in sulphur conditions were induced via a reciprocal transplant of lichen thalli between urban/polluted and marine/unpolluted locations, the level of pollution being a function of the concentration and origin of the atmospheric sulphur.

The question as to whether epiphytic lichens are able to respond quantitatively to changes in sulphur conditions has already been partly answered (Wiseman and Wadleigh, 2002), and so the interest of this study lay rather in quantifying this response and comparing it for both directions of change. Some studies have investigated qualitative lichen responses to improvements in air quality (*e.g.* increases in biodiversity and/or lichen numbers), but we are not aware of any study in which a quantitative response was investigated. Furthermore, the extent of background variation (both natural and anthropogenic) and seasonality in sulphur isotopic composition and concentration remains to be determined.

The general purpose of this study is to investigate the response of the epiphytic pendulous lichen *Alectoria sarmentosa* to changes in the concentration and isotopic composition of atmospheric sulphur as characterized by changes in the lichen thallus of these parameters. Specific objectives include: i) to determine whether the act of transplanting lichen material affects the response of lichens to changes in the concentration and isotopic composition of atmospheric sulphur, ii) to assess the seasonality of the abundance and isotopic composition of atmospheric sulphur, and iii) to determine whether the speed and magnitude of change vary with the direction of transplant (*e.g.* from "polluted" to "unpolluted" or vice versa).

It is anticipated that relocating lichens withing the same general area will not affect their response to sulphur conditions. Some background (natural and/or anthropogenic) variation is expected at both sites and is expected to be related mostly to anthropogenic sources at the polluted sites and natural sources (sea spray) at the unpolluted site. It is

expected that the sulphur content and isotopic composition of transplanted lichens will change gradually under the new conditions until becoming indistinguishable from those measured in locally-growing lichens. The response of lichens originating from the polluted site is expected to be slower than that of lichens originating from the unpolluted site, perhaps as a consequence of physiological damage sustained from prolonged exposure to high levels of sulphur dioxide.

From a biological standpoint, it would be interesting to consider the influence of the change of sulphur isotopic composition and concentration on lichen physiology and growth. As well, it is likely that additional variables, such as lichen age, play a role in the response mechanism of lichens. Photosynthetic rates have been found to be greater in younger portions of lichen thalli (Maguas and Brugnoli, 1996). Such considerations are, however, beyond the scope of this study.

## 1.2 The sulphur system

Understanding the global sulphur cycle is of primary importance, especially in view of the significant contribution of anthropogenic sources to the cycle. This section includes a brief overview of the global biogeochemical sulphur cycle with emphasis on the atmospheric component and anthropogenic contributions. The presence of sulphur in the biosphere is discussed, and a brief section on sulphur isotopes is included.

### 1.2.1 The global sulphur cycle

Sulphur is the fourteenth element in abundance in the Earth's crust, occurring to the extent of 0.047% (Grinenko and Ivanov,1983). It exists in nature in five valence states (-2, 0, +2, +4 and +6 ) and, although not required in large quantities, is essential to life for both plants and animals. The biogeochemical sulphur cycle has changed considerably throughout the planet's history, mainly due to the appearance of new metabolic pathways and changes in their importance (Schlesinger, 1991), but its present state reflects extreme human-induced perturbations.

Three major global reservoirs of sulphur can be identified, these are: reduced sulphide of sediments, oxidized sulphate of evaporites and other sediments, and seawater sulphate (Holser *et al.*, 1989; Schlesinger, 1991). The sulphur cycle is driven by transformations between the different valence states, which are accomplished in part through inorganic processes and in part through microbial activity (Schlesinger, 1991). The release of reduced sulphur compounds into the oxidizing atmosphere of our planet leads to their oxidation to sulphur dioxide ($SO_2$) and methane sulphonic acid ($CH_3SO_3H$ or MSA)(oxidation state +4), and ultimately to sulphuric acid ($H_2SO_4$, oxidation state +6). The oxidation state of +6 is the thermodynamically stable form of sulphur in the presence of oxygen. Microbial reduction processes complete the cycle by transforming the oxidized sulphur back to its reduced forms.

The three basic reservoirs and the interactions between them are illustrated in Figure 1.1. Weathering on the continents mobilizes the sulphur bound in pyrite minerals and

evaporites, while river flow carries it to the oceans. The fate of marine sulphate is either to be precipitated as sulphate in evaporite deposits or to be reduced by biological processes and deposited as pyrite in marine sediments (Holser *et al.*, 1989). The biological reduction process is carried out by anaerobic sulphur-reducing bacteria of the genera *Desulfovibrio* and *Desulfotomaculum* (Roy and Trudinger, 1970). The overall reaction can be represented by:

$$SO_4^{2-} + CH_2O \rightarrow S^{2-} + CO_2 + 2H_2O$$

where the $CH_2O$ represents any degradable organic carbon and the $S^{2-}$ represents any completely reduced sulphide (Holser *et al.*, 1989). This process is termed "dissimilatory sulphate reduction" (DSR) and can be thought of in terms of a process similar to denitrification, with the $SO_4^{2-}$ acting as an alternative electron acceptor during the oxidation of organic matter. The term "dissimilatory" implies that the sulphate is not used as a nutrient by the bacteria carrying out the reaction, but rather as a means of obtaining the necessary energy for metabolic functions.

Let us now consider the global sulphur cycle in more detail. Figure 1.2 illustrates the magnitude and direction of the major sulphur fluxes between ocean, land, and atmosphere before the industrial era (a) and at present (b). The lithosphere is the main source of mobile sulphur, and the ocean is the final sink. The minerals anhydrite ($CaSO_4$) and gypsum ($CaSO_4 \cdot 2H_2O$) found in oceanic deposits are two of the most geochemically important evaporite minerals. These contain sulphur in the oxidized form of sulphate (+4), which is the third ion in importance in ocean water ($[SO_4]$ = 2712 mg/kg; Holland, 1978) after

sodium and chloride. The sulphur supplied to the cycle by continental rocks is mainly in the form of pyrite minerals, where it exists in its most reduced form; sulphide (−2). The weathering and erosion of evaporite and sulphide minerals contribute to the natural river load of $SO_4$.

Mobilized sulphur eventually reaches the oceans as sulphate (via river flow or from precipitation originating above land), where it can be taken out of the system through the processes mentioned above, or recycled. Most of the sulphur recycled to the atmosphere is in the form of sulphate aerosols, or sea spray (Brimblecombe *et al.*, 1989). These particles enter the atmosphere in the form of tiny droplets that have become airborne as a result of the bursting of bubbles at the surface (MacIntyre, 1974; Wu, 1981). The salt left behind when the water evaporates from the bubbles crystallizes and forms the sea salt aerosols, which crudely approximate the composition of seawater (Glass and Matteson, 1973). Sea spray sulphates are found everywhere in the oceanic atmosphere and along the coasts.

Sulphur can also be transferred from the oceans to the atmosphere through other processes, such as the production of biogenic gases, volcanic eruptions and the release of S compounds at hydrothermal vents. The major annual movement of sulphur through the atmosphere occurs from land to sea. This is believed to be a result of human influences on the sulphur cycle, without which the net transport of sulphur would carry a small amount of S in the reverse direction (Brimblecombe *et al.*, 1989; Schlesinger, 1991). Anthropogenic S emission at present accounts for approximately 55% of total sulphur input to the continental atmosphere (Brimblecombe *et al.*, 1989).

1.2.1.1 Sulphur in the atmosphere

The atmosphere is a very mobile system in which most processes take place in no more than a few days. Sulphur enters the atmosphere in gaseous or particulate form from a variety of sources. The major natural fluxes influencing the global atmospheric sulphur budget are: i) biogenic emissions from coastal regions, the open ocean and the land, ii) aeolian weathering of sulphates, iii) sea salt sulphate from the ocean, and iv) volcanic exhalations. Table 1.1 lists the major sulphur gases and their characteristics.

Biogenic sources of sulphur have been estimated to constitute as much as 50% of the total atmospheric burden (Kellogg *et al.*, 1972; Ryaboshapko, 1983). These emissions occur predominantly in the form of reduced sulphur compounds. They can be grouped in the following way: emissions from vegetation, emissions from wetlands, emissions from land, and emissions from oceanic environments. Firstly, because sulphur is a necessary element for plant growth, it can be released directly during the decomposition of organic matter. Sulphur is known to be volatilized from living plant leaves, as well as decaying leaves, and to be released by bacteria and fungi during plant decomposition. Some plants are also known to emit hydrogen sulphide ($H_2S$), dimethylsulphide ($CH_3SCH_3$ or DMS), carbonyl sulphide (COS) and carbon disulphide ($CS_2$) (Aneja and Cooper, 1989). Secondly, sea marshes and the tidal flats of marine environments are areas of intense biological activity that serve both as sources and sinks for a number of sulphur compounds. Because of the relatively high concentrations of sulphate in marine waters, sulphur plays an important role in biological processes in these environments, notably as the major electron acceptor for respiration in

anoxic sediments (Ingvorsen and Jørgensen, 1982). Although $H_2S$ is the major sulphur product of this process, other volatile sulphur compounds, such as DMS, DMDS (dimethyldisulphide; $CH_3S_2CH_3$), $CH_3SH$ (methyl mercaptan), COS and $CS_2$ have been identified in such environments (Aneja *et al.*, 1979; Hill *et al.*, 1978). Thirdly, the emission of volatile sulphur compounds such as DMS, $H_2S$, $CS_2$ and COS has been measured on land, especially in wetland areas, but also from bare soils (Goldan *et al.*, 1987). And lastly, the oceans are a major source of biogenic sulphur for the atmosphere, especially DMS, which is produced by benthic and mostly planktonic marine algae (Andreae and Barnard, 1984). DMS is the major biogenic gas emitted from the ocean and represents 50% of all biogenic gases emitted to the atmosphere (Ferek *et al.*, 1986). DMS, which is oxidized to a sulphate aerosol in the atmosphere, is believed to be the major source of cloud-condensation nuclei over the oceans and to play an important role in the regulation of the Earth's climate (Charlson *et al.*, 1987).

The second most important flux in the atmospheric component of the sulphur cycle is the aeolian weathering of sulphates from the continental surface. This flux is difficult to quantify and may vary greatly in time and space. Arid regions, and especially areas where dust storms are frequent, may contribute the largest portion of aeolian sulphur material to the atmosphere (Grinenko and Ivanov, 1983). At present, the contribution of this source to the atmospheric sulphur budget is estimated at less than 6% of total S emissions (Brimblecombe *et al.*, 1989) and is thought to have increased significantly as a result of human activities that increase erosion, such as the development of grazing, intense pasturing

in dry regions, irrigative farming in arid zones, etc. (Brimblecombe *et al.*, 1989).

The contribution of sea spray sulphates to the global sulphur budget is uncertain. Brimblecombe *et al.* (1989) estimate its annual contribution to the cycle as 144 Tg (S), which represents approximately 42% of the total sulphur input to the atmosphere. Only a very small proportion of the seawater sulphate passes over land (~10%), a transfer which is possible only in coastal locations (Moss, 1978; Whelpdale, 1992).

Finally, volcanic exhalations contribute predominantly $SO_2$ and $H_2S$, with some $SO_3$ and various sulphates (Kellog *et al.*, 1972). Although most of the sulphur on the Earth's surface originated from outgassing of deep crustal and mantle regions, the contribution of volcanoes to the present sulphur budget is quite small, with estimates as low as 2% (Nielsen, 1974). $H_2S$ is also emitted in significant amounts by hydrothermal vents in the ocean (Brimblecombe *et al.*, 1989).

1.2.1.1.1 Sulphur deposition processes

Compounds in the atmosphere can be brought to the surface through a variety of mechanisms. Those mechanisms for which the mode of deposition is precipitation are termed "wet deposition processes", while those that do not involve precipitation are referred to as "dry deposition processes".

Sulphur removal from the atmosphere by wet deposition can occur in several ways. Sulphur gases can be removed during uptake into raindrops within clouds (in-cloud scavenging), uptake into raindrops as they are falling to the ground beneath the clouds (sub-

cloud scavenging) (Ryaboshapko, 1983), or they can be adsorbed onto frozen precipitation elements (Whelpdale, 1992). Sulphate particles are efficient cloud condensation nuclei (CCN) and can thus be incorporated into precipitation or scavenged by cloud droplets and falling drops (Whelpdale, 1992). The efficiency of sulphur removal by wet deposition processes depends on their form and on characteristics of the precipitation (*i.e.* type, intensity, duration, frequency). For aerosol particles with diameters between 0.1 and 1 μm, the primary removal mechanism is precipitation scavenging (Brasseur *et al.*, 1999). Particles with diameters of 10 μm or more may be removed by gravitational sedimentation. Smaller particles and gases are more efficiently brought back to the surface by turbulent atmospheric motions. The actual uptake of these smaller particles may be accomplished by chemical reaction, dissolution, adsorption and other mechanisms (Whelpdale, 1992).

1.2.1.2 Anthropogenic contributions

Technological developments have altered the global biogeochemical sulphur cycle, but the anthropogenic influence takes place in many ways, some of which are not easily quantified.

The most substantial disturbance to the sulphur cycle arises from the combustion of sulphur-containing fuels for energy purposes. Sulphur may be present in all fuels of biogenic origin, albeit with great variation. The purest of fuels with regards to sulphur content is natural gas, with an average concentration of 0.05% S (by weight) (Ryaboshapko, 1983). In general terms, coal is the type of fuel which contains the most sulphur by weight, with a

world average of 2.2% (Bhatia, 1978). Sulphur exists in natural gases and oils as hydrogen sulphide and organic compounds, whereas it exists in coals as organic compounds, pyrites, and sulphate (Ryaboshapko, 1983).

During fuel combustion, sulphur is oxidized to sulphur dioxide ($SO_2$) and partially to sulphur trioxide ($SO_3$), and released into the atmosphere. It is generally accepted that 95% of the sulphur in fuel is released into the atmosphere upon combustion (Kellogg *et al.*, 1972), of which 96% is in the form of $SO_2$ and 4% in the form of $SO_3$ (Kiyoura *et al.*, 1970). Most of the sulphur dioxide emitted is deposited locally in precipitation and dryfall; the remaining undergoes long range transport (LRT) (Schlesinger, 1991). Sulphur dioxide may be oxidized in the atmosphere in the gas phase, on the surface of soil particles, and in the liquid phase of droplets in clouds and fog (Ryaboshapko, 1983). Oxidation in the gas phase is by far the dominant process, and is attributable primarily to reaction with the hydroxyl radical (OH) to form $SO_4^{2-}$ (Cox and Sheppard, 1980). Because the concentration of the hydroxyl radical may vary according to cloudiness, time of day, season and latitude (intensity of solar radiation), the rate of $SO_2$ oxidation may be extremely variable.

Other anthropogenic activities that significantly affect sulphur cycling through the emission of sulphur gases are: ferrous and non-ferrous ore smelting, oil processing, and sulphuric acid production (Ryaboshapko, 1983). Anthropogenic sulphur emissions exhibit daily, weekly and seasonal variation.

Several other processes by which human activities increase sulphur mobilization should also be considered, in spite of the fact that these are not directly linked to industrial

emissions. These are: i) increased mobilization of sulphate from rocks and soils through mining and agricultural practices, leading to the augmentation of sulphur concentration in river runoff, ii) increased aeolian emission of sulphate-containing dust to the atmosphere from dry land surfaces, caused by farming and animal husbandry practices, and by increased exposure of salty lake sediments, iii) the increased production of volatile sulphur compounds in coastal seawater as a result of fertilization by nitrate, phosphate and other organic materials, and iv) changes in the rates of chemical transformation of S compounds in the atmosphere caused by modifications in the chemical climate (such as the abundance of hydroxyl radicals over industrialized regions) (Brimblecombe *et al.*, 1989).

1.2.1.3 Sulphur in the biosphere

Sulphur is required by living systems and can be found in a wide variety of compounds, but only a few of these are considered to be required for normal cell function (in plants). These are: the amino acids cysteine and methionine, glutathione, thiamine, vitamin B, biotin, ferrodoxin, lipoic acid, coenzyme A (Krouse *et al.*, 1991). As a general rule, 90% of the sulphur in plants is contained in the S-containing amino acids (Blair, 1979).

Most of this sulphur is obtained through the absorption of sulphate from the soil solution, but various sulphur compounds present in the atmosphere may be deposited on vegetation surfaces (including lichens), where they can be oxidized when they encounter water (Taiz and Zeiger, 1998). Higher plants are also able to metabolize some gaseous sulphur compounds through their stomata (Taiz and Zeiger, 1998). Most plants have sulphur

contents of 0.1 - 1.5% on a dry weight basis (De Kok, 1990). Assimilatory sulphate reduction (ASR) is the sulphur absorption process used by autotrophs. During this process, absorbed sulphate (+6) is reduced to form the thiol groups (−4) of sulphur-containing organic compounds. There appear to be two pathways for ASR: the first is used by most oxygen-evolving photosynthesizers (all eukaryotic algae, some prokaryotic cyanobacteria, higher plants) and utilizes adenosine-5'-phosphosulphate (APS) as a sulphate donor for reduction; the second is used by organisms that lack oxygen-evolving photosynthesis (yeast, some bacteria -such as *E. coli*-, some cyanobacteria), with 3'-phosphoadenosine-5'phosphosulphate (PAPS) as the sulphate donor. (Schiff, 1983).

The APS pathway involves the uptake of sulphate from the external environment, its activation with ATP (adenosine triphosphate), its transfer to a carrier molecule, and its reduction to sulphite ($SO_3^{2-}$; +4), and subsequently to sulphide ($S^{2-}$; −2)(Trust and Fry, 1992). It is represented in Figure 1.3 by the black arrows.

The activation process with ATP is necessary due to the stability of $SO_4^{2-}$ (Taiz and Zeiger, 1998). The products of the activation reaction are adenosine-5'-phosphosulphate (APS) and pyrophosphate ($PP_i$). The reaction is energetically unfavourable, and so the products must be rapidly converted: $PP_i$ is hydrolyzed to organic phosphate ($P_i$); APS is thought to react with ATP to form 3'-phosphoadenosine-5'phosphosulphate (PAPS), before being reduced to sulphite, and subsequently to sulphide (Taiz and Zeiger, 1998). The sulphide is eventually incorporated into the amino acid cysteine, which is the precursor to the amino acid methionine (Schiff, 1983; Taiz and Zeiger, 1998).

The PAPS pathway is the main reductive pathway of bacteria and cyanobacteria (Figure 1.3: white arrows). In the reaction sequence of this assimilatory pathway, the internalized sulphate is further activated to PAPS by another ATP molecule before being transferred to a thiol carrier ($tr(SH_2)$) and reduced to the oxidation state of −4 in sulphite. The last step involves the further reduction of sulphite to sulphide (−2) and is carried out by reduced ferredoxin (Andreae and Jaeschke, 1992; Trust and Fry, 1992).

Among the biological processes that involve sulphur is a sulphur oxidation process utilized by some bacteria. Autotrophic bacteria belonging to the genus *Thiobacillus* are considered to be the most important group of these sulphur-oxidizing organisms. These are able to use elemental sulphur and incompletely oxidized inorganic compounds as specific electron donors for the assimilation of carbon dioxide (Ralph, 1979).

Dissimilatory sulphate reduction is a process in which the sulphate ion is used by some bacteria as an alternative electron donor under anaerobic conditions (section 1.2.1).

### 1.2.2 Sulphur isotopes and isotope fractionation

Originally, the aim of isotope abundance measurements was to identify natural isotopes of elements and to quantify their natural abundance. In the more recent years, studies have focussed on natural stable isotopes (mainly C, H, O, N and S) to interpret geochemical changes in nature.

Sulphur has 16 isotopes, four of which are stable: $^{32}S$ (95.02%), $^{33}S$ (0.75%), $^{34}S$ (4.21%) and $^{36}S$ (0.02%). Due their greater relative abundance, $^{32}S$ and $^{34}S$ are those

normally used in stable isotope research. The sulphur isotopic composition of a given material is stated relative to a reference material and expressed as units of per mil (x/1000: ‰). The historical reference material for relative isotope ratio measurements of sulphur isotopes was an iron sulphide (FeS) from the Cañon Diablo iron meteorite, and was believed to represent the primordial ratio of $^{34}S$ to $^{32}S$ (1:22.22). Because of large variability in $\delta^{34}S$ (up to 0.4‰; Beaudoin *et al.*, 1994), its use has been discontinued. The reference material now used is Vienna CDT (V-CDT). It has a sulphur isotopic composition of 0‰. The isotopic composition of a sample is related to that of a reference material according to the following equation:

$$\delta^{34}S\ (‰) = \left[ \frac{^{34}S/^{32}S_{sample}}{^{34}S/^{32}S_{reference}} - 1 \right] \bullet 1000$$

where $^{34}S/^{32}S$ represents the ratio of $^{34}S$ to $^{32}S$ isotopes in the reference material and sample. A positive $\delta^{34}S$ value indicates an enrichment in the heavy isotope $^{34}S$, whence a negative value denotes enrichment in the light isotope $^{32}S$.

"Isotopes effects" is the term given to the differences in chemical and physical properties that arise as a result of differences in the atomic mass of an element (Hoefs, 1987). These "effects" can lead to the differential exchange of isotopes of a given element in a reaction, a principle known as "isotope fractionation". The main phenomena that can produce isotopic fractionation are isotope exchange reactions and kinetic processes. Isotope exchange reactions are processes in which the isotope distribution between different chemical substances, different phases or individual molecules, changes, but without causing

any change in the chemical system itself. Such reactions can be viewed as a case of general chemical equilibrium, with heavier isotopes being preferentially accumulated in the molecule with the stronger bonds, or in the case of different phases, in the phase with the lower vapour pressure (solid>liquid>gaseous) (Hoefs, 1987).

Kinetic effects leading to isotope fractionation occur when "the rate of a chemical reaction is sensitive to atomic mass at a particular position in one of the reacting species" (Hoefs, 1987). Isotope measurements taken during unidirectional chemical reactions always demonstrate an enrichment of the lighter isotope in the end products of the reaction.

In the case of sulphur, most of the natural range of isotopic composition is between −40‰ to +40‰ relative to CDT. The isotopic compositions of the main atmospheric sulphur species are shown in Figure 1.4. Stable sulphur isotope measurements constitute a powerful tool in the investigation of sources of anthropogenic sulphur in the environment in cases where these differ significantly in their signatures from those of the natural sources.

1.2.2.1 Sulphur isotope fractionation in biological systems

Assimilatory sulphate reduction by autotrophs generally results in small isotope shifts, with $\delta^{34}S$ that are typically 1.5‰ lighter than the sulphate source. This has been verified for algae and aquatic plants (Mekhtiyeva, 1971) and epiphytic lichens and mosses, which use atmospheric S as a sulphur source (Krouse, 1977; Winner *et al.*, 1978; Case and Krouse, 1980). On the one hand, the initial step in the assimilatory pathway of sulphate reduction -the uptake of sulphate in the cell- is expected to result in very little isotope

fractionation (Figure 1.3) (Trust and Fry, 1992). The same can be said for the activation steps involving ATP and the transfer to a carrier molecule. On the other hand, large fractionation is expected for the reduction steps involving the breaking of sulphur-oxygen bonds (Trust and Fry, 1992). It is thought that the rate of $SO_4^{2-}$ reduction in plants is limited either by the uptake, or the activation of sulphate, as the overall fractionation observed is small, which would not be the case were the reaction limited by one of the reduction steps (Trust and Fry, 1992).

Because higher plants may be able to use sulphur gases in the atmosphere as a sulphur source in addition to dissolved sulphate from the soil solution, it has been suggested that the differing isotopic composition of sulphur in various plant parts (*e.g.* roots as opposed to leaves) may reflect differences in sulphur source. For example, conifer needles and deciduous leaves have $\delta^{34}S$ values between those of soil sulphate and atmospheric sulphur gases (Krouse *et al.*, 1991; Figure 1.5). This has also been demonstrated in a study by Krouse *et al.* (1984) for the moss *Polytrichum juniperinum* (Figure 1.6). Measurements for the upper portion of the moss yielded $\delta^{34}S$ values near + 20 ‰, hence very close to the $\delta^{34}S$ of the source (West Whitecourt Gas Plant, Alberta: $\delta^{34}S$ = 22‰). The root sections, however, had a $\delta^{34}S$ of +13‰, consistent with the isotopically lighter sulphur present in the surrounding humus. Lichens, on the other hand, have no roots and tend to have $\delta^{34}S$ values consistent with atmospheric S (Krouse, 1977; Krouse *et al.*, 1984). Although ASR by plants in normal conditions rarely results in isotope shifts that exceed a few per mil, there is evidence that abnormal nutritional factors (*e.g.* sulphur-stress in high concentrations of $SO_2$)

may give rise to larger isotope shifts. For example, Winner *et al.* (1981) found that the $H_2S$ emitted by plants as part of a protection mechanism against sulphur dioxide stress (DeCormis, 1968; Spaleny, 1977; Wilson *et al.*, 1978) was depleted in $^{34}S$ compared to the sulphur source, a situation which resulted in an enrichment in $^{34}S$ in the plant. Studies by Case and Krouse (1980) and Krouse *et al.* (1984) yielded similar results.

## 1.3 Lichens

### 1.3.1 The lichen symbiosis

Lichens are long-lived perennial organisms characterized by a symbiotic association between mycobiont (fungus) and photobiont partners (eukaryotic alga; phycobiont or cyanobacteria; cyanobiont) (Hale, 1974). Lichens are amongst the most widely distributed eukaryotic organisms in the world, with approximately 13 500 known species, encompassing 20% of all known species of fungi (Galun, 1988). The photobiont and mycobiont partners live in a close, mutually beneficial association, forming a new entity with usually very little resemblance to either one of its components (Galun, 1988). The fungal partner typically accounts for 90% of the total lichen mass, and so the fungus provides the taxonomic definition of a lichen species (Galun, 1988). The mycobiont provides structure and protection from the elements to the photobiont, while in return being provided with photosynthates (polyols in the case of green algal lichens; glucose in the case of cyanolichens) (Nash, 1996). A cyanobiont partner also provides nitrogen fixed from the atmosphere to the mycobiont. (Honegger, 1991). Some associations may include organisms

from as many as three different kingdoms; a fungus, an alga, and a cyanobacterium. Some 400 such species of lichens have been recognized thus far (Galun, 1988).

The evolution of lichens is subject to great uncertainty among lichenologists. Some have hypothesized that lichen components (green algae, cyanobacteria and fungi) evolved from lichens, but it is generally assumed that the opposite situation actually occurred. There is very little evidence of lichens in the fossil record, but this may well be due to lack of preservation rather than absence (Nash, 1996). What some consider to be the oldest lichen fossil dates from the Mesozoic epoch, with many more from the Cenozoic (Smith, 1921).

### 1.3.2 Lichen growth forms and morphology

Much variability exists in the anatomy and morphology of lichens. Above all, the anatomical and morphological configurations must serve to facilitate the coexistence of the symbiotic partners and the exchange of metabolites (Jahns, 1988), but there are an infinite number of possibilities in shapes and tissue arrangement that may serve this purpose. The lichen thallus is the structure that differentiates between lichenized and non-lichenized fungi (Jahns, 1988). Lichen thalli are traditionally classified into three growth forms: crustose, foliose and fruticose (Hale, 1974), with some intermediates. The arrangement of the different tissues and the degree of attachment to the substrate are the distinguishing elements used in the classification. It is important to note that although such a classification system is undoubtedly useful, there exists no clear-cut definition as to where exactly the boundaries between the growth forms lie. The following includes a brief description of basic

morphological components and lichen growth forms.

Within the thallus, the main tissue subdivisions are into upper cortex, photobiont layer, medulla and lower cortex. Heteromerous lichen species have stratified thalli with clearly defined algal layer and medulla (and cortex in some), whereas homoeomerous species, which are considered to be primitive lichens, show very little anatomical differentiation (Jahns, 1988). The basic building blocks for the lichen thallus are elongate cellular threads, called hyphae (Ahmadjian, 1967). The vast majority of lichens possess an upper cortex, which is usually 10-15 um thick and composed of more or less heavily gelatinized hyphae cemented together (Ahmadjian, 1967). The cortical tissues may serve different functions, including: mechanical protection, modification of energy budgets (Kershaw, 1985), anti-herbivore defence (Reutiman and Scheidegger, 1987) and protection of the photobiont against excessive light (Büdel, 1987; Jahns, 1988). The algal layer is formed by the upper part of the medulla, which usually occupies the centre of the thallus structure and is composed of loosely interwoven hyphae threads (Ahmadjian, 1967). Figure 1.7 shows cross-sections of lichens from the crustose (a), foliose (b) and fruticose (c) growth forms.

The crustose lichens are flattened, with the entire lower surface growing on the substrate, and cannot be loosened without damage (Jahns, 1988). A transect of a crustose lichen from top to bottom reveals an upper cortex, an algal layer, and a medulla. Together, the endolithic (growing inside rocks) and endophloeodic (growing inside wood) varieties comprise the majority of the crustose growth type (Büdel and Scheidegger, 1996), which

includes more than 80% of all lichen species (Büdel and Scheidegger, 1996). The squamulose growth type represents a transition between the crustose and foliose lichens. It is characterized by scale-like overlapping lobes that are not fully attached to the substrate, but raised above it (Jahns, 1988).

Foliose and fruticose lichens, collectively called the 'macrolichens', are loosely attached to and easily removed from the substrate (Jahns, 1988). Foliose or leaf-like lichens typically have a stratified organization, with easily distinguishable upper and lower surfaces. Some species connect to the substrate using root-like structures called rhizines (Jahns, 1988). Both a lower and upper cortex can be identified in a microscopic vertical cut.

The fruticose lichens grow erect or pendent, and the thalli possess no clearly distinguishable upper or lower surfaces. The different tissues are, as in the foliose type, layered, but the thalli are built in three dimensions: the cortex envelops the thallus stalks or filaments, while the algal layer is located in the centre. The thallus of the fruticose lichens can be strap-shaped (resembling foliose thalli; built both radially and dorsiventrally) or cylindrical (radial construction) (Jahns, 1988). Many fruticose lichens are epiphytic, thus dependent upon other vegetal organisms for mechanical support, but not for nutrients. Epiphytic lichens derive moisture and nutrients from dry and wet deposition, with little or no influence from the substrate (Sloof and Wolterbeek, 1993).

Some lichen genera develop a two-fold thallus differentiated into a horizontal part (*thallus horizontalis*) and an erect part (*thallus verticalis*) (Jahns, 1988). The horizontal component of the thallus can be of the crustose or foliose types, while the vertical

component is typically of the fruticose type. The gelatinous lichens, which are composed of mycobiont and cyanobiont partners, do not represent, *per se*, a lichen growth form, as these may exhibit the crustose, foliose, and fruticose growth forms. The gelatinous quality of these lichens exists only when the lichens are wet, and is a result of water absorption in the gelatinous sheaths cyanobacteria (Jahns, 1988).

### 1.3.3 Lichen nutrition

#### 1.3.3.1 Nutrient sources

Lichens derive the macro- and micronutrients necessary for their growth and survival from the atmosphere and/or the substrate (Nash, 1996). Atmospheric deposition to lichens occurs by wet (precipitation and occult precipitation) and dry (sedimentation, impaction and gaseous absorption) deposition mechanisms (Knops *et al.*, 1991). Occult precipitation (principally fog and dew) is particularly important to lichens both for nutritional reasons and as a source of moisture, as the concentrations of nutrients (and contaminants) may be substantially higher in occult precipitation compared to rainfall (increased dilution) (Nash, 1996).

Many lichens, especially those of the crustose and foliose types, occur on soils or rocks and may be in intimate contact with lithic sources of nutrients (Jahns, 1988). Lichens can effect the weathering of rocks and soils by mechanical or chemical means, leading to the mobilization of nutrients, followed by uptake into the lichen. It is not surprising then that many lichen species appear to be confined to particular rock or sediment types, and even

species of trees (Wirth, 1972; Roux, 1981). A proportion of the total nutrient intake may originate from aeolian dust carrying relatively large amounts of Al, Fe, Sc, Ti and other elements of lithic origin (Richardson, 1992). Epiphytic lichens may as well be affected by the dynamics of nutrient processing in the canopy, where elements leached from the foliage may become readily available.

1.3.3.2 Accumulation mechanisms

As a result of the absence of a root system and protective structures (*i.e.* stomata, waxy layer, cuticle), the exchange of elements and nutrients in lichens occurs across the entire surface (see section 1.4.1). Three major mechanisms by which nutrient or contaminant accumulation occurs are: ion exchange in cell walls, intracellular uptake, and particulate entrapment.

Ions absorbed by lichens via the ion exchange process are typically positively charged metallic ions (Richardson, 1992). These bind to sites on the cell walls of both the fungi and algae (Xue *et al.*, 1988; Tyler, 1989), probably on carboxylic groups that are part of proteins (Richardson *et al.*, 1985). Once bound to the cell wall, the ions may be displaced by others with a greater affinity (Richardson and Nieboer, 1981). Ion-competition experiments conducted by Nieboer and Richardson (1980) determined that the affinity of ions for exchange sites increases in the following sequence: monovalent class A < divalent Class A < borderline divalent < divalent Class B. Anion uptake by lichens (mostly anions of uranium) has been investigated to a more limited level; anion exchange sites have not

been identified thus far.

Intracellular uptake consists of less than 10% of total uptake (Brown and Beckett, 1985) and is assumed to involve energy expenditure, as has been demonstrated for the intracellular uptake of phosphate (Farrar, 1976).

Particulate trapping may be one of the ways by which lichens are able to accumulate elements beyond their metabolic needs without effecting significant damage to metabolic functions. Considerable intracellular space exists within lichens (*e.g.* estimated at 18% for *Xanthoria parietina*: Collins and Farrar, 1978) and the presence of trapped particles within these spaces has been demonstrated with scanning electron microscope procedures (Garty *et al.*, 1979: Johnsen, 1981; Jones *et al.*, 1982; Purvis *et al.*, 2000). Insoluble particulates from metal-rich emissions become entrapped in the expanding hyphae of the lichen, much in the same way as particulates derived from the soil or substrate may become part of the thallus structure (Richardson, 1992).

1.3.3.3 Water relations

Lichens are poikilohydric organisms, thus their water status varies with their surrounding environment (Nash, 1996). The uptake of water, which may come from a variety of sources, from liquid precipitation and collected runoff to fog and dew, is generally regarded as being passive. Although biological variables such as thallus structure and anatomy may contribute significantly to the process of water absorption and evaporation, the dynamics of water movement in lichens are largely controlled by physical processes

(Rundel, 1988; Nash, 1996). This passive relationship translates into a question of balance between absorbing water to maintain thallus moisture content (thus maximizing rates of net photosynthesis) and restricting rates of water loss.

One way for lichens to maximize photosynthetic production lies in having high surface to volume ratios, as in the fruticose and the foliose growth forms. This can be achieved by changes in the growth form or branching patterns (Rundel, 1988), but is profitable only when water availability is not an issue. For example, whereas temperate forest areas with high amounts of precipitation throughout the year (and high light levels) are dominated by the macrolichens, xeric habitats support mostly crustose varieties ( Rundel, 1980; Kantvilas *et al.*, 1985; Rundel, 1988).

Typically, lichens in the air-dry state have water contents of less than 15-30% on a weight basis (Nash, 1996), but their saturated moisture contents are quite high (120-200% of the thallus dry weight for typical fruticose and foliose lichens with phycobionts), especially those with cyanobionts (250-400% for some genera, 400-1300% for some of the gelatinous lichens) (Blum, 1973). Dry thalli do not show any detectable $CO_2$ exchange (Rundel, 1988), but 1-2 minutes following rehydration from a desiccated state, non-metabolic release of $CO_2$ begins (Smith and Molesworth, 1973). This is followed by a period of resaturation respiration, during which the levels of $O_2$ consumption and $CO_2$ release exceed normal base levels for hydrated thalli; this stage may last from one hour to many days (Link and Nash, 1984), until falling ambient humidity levels cause the lichens to dessicate. In the desiccated state, photobiont cells loose their turgor and become contorted

and smaller in size (Brown *et al.*, 1987; Büdel and Lange, 1991), gas exchange ceases and the cell membranes become partially leaky as a result of the loss of integrity (Brown and Brown, 1990). Most lichens are resistant to dessication, and many are able to maintain positive rates of photosynthesis with thallus moisture in equilibrium with air at 80% humidity (Rundel, 1988), a trait which sets them apart from most vascular plants and bryophytes, which are incapable of hydrating beyond the moisture compensation point with aerosol water only (Rundel, 1988). Tolerance to dessication varies widely between species and is believed to be linked to the presence of glutathione disulphide (GSSG), a product of the oxidation of glutathione (γ-glutamyl-cysteinyl-glycine), which plays a significant role in biological functions in catalysis, synthesis and transport (Bergman and Rennenberg, 1993; Meister, 1995). The accumulation of GSSG and protein-bound gluthianone apparently protects thiol groups from dessication-induced oxidative injury in dessication tolerant plants and lichens (Kranner and Grill, 1997).

### 1.3.4 Lichen reproduction

The reproduction of a symbiotic organism may be regarded as somewhat complex, if one considers that if the two partners are disseminated separately, the symbiosis must be reestablished each time. While a large proportion of foliose and fruticose lichens reproduce by vegetative means (*i.e.* propagules including both members of the association), most of the crustose species produce large amounts of fungal spores, apparently depending upon resynthesis for dispersal (Galun, 1988). But clearly, a prerequisite for resynthesis following

spore release is the existence of the algal partner (Trebouxia, comprising 50-75% of all lichen photobionts) in a free-living state, something which has only recently been unequivocally proven (Galun, 1988). In lichens, usually only the mycobiont expresses full sexual reproduction (Büdel and Scheidegger, 1996).

Vegetative reproduction implies the dispersal of symbiotic propagules (*i.e.* containing both partners). Of the vegetative propagules, isidia and soredia are the most important (Büdel and Scheidegger, 1996). Isidia are finger-like structures, ranging in height from 30 μm to 1mm, often cylindrical and simple or branched that are present on the thallus surface (Büdel and Scheidegger, 1996). Soredia consist of a few photobiont cells enveloped in a loose, spherical cover of hyphae, ranging from 20 to 50 μm in diameter, developing on the thallus surface (Büdel and Scheidegger, 1996). Lichens in the dessicated state are very brittle and crumble easily, releasing fragments that may develop into new thalli (Büdel and Scheidegger, 1996). While most vegetative reproduction occurs via the dispersal of symbiotic propagules, some lichens are known to release actual thallus fragments -under high winds or after trampling by animals- that may develop into new individuals (*e.g.* *Bryoria*, *Ramalina* and *Cladonia*; Büdel and Scheidegger, 1996).

### 1.3.5 Alectorioid lichens

*Alectoria sarmentosa* ("Witch's hair") is part of a group of lichens commonly known as the beard or hair lichens and is the most common of the tree-dwelling species of the *Alectoria* genera, which comprises seven North American species (Brodo *et al.*, 2001).

Lichens of the genera *Alectoria* are shrubby to pendent epiphytic fruticose lichens, with slender, hair-like rounded branches. *Alectoria* species grow attached to bark and wood or on the ground in well-lit situations (Brodo *et al.*, 2001). Thallus colour usually ranges from pale greenish yellow to gray to black (rare); the yellow-green tones are a result of the presence of usnic acid (Brodo and Hawksworth, 1977). Although there are some exceptions, *Alectoria* can be thought of as a cold climate genus, with most species found in northern or mountainous areas (Brodo and Hawksworth, 1977). *Alectoria* lichens are usually found on the acidic bark of coniferous trees, although it is not known whether this is due to an actual preference for the bark of these trees, or a result of climatological factors. It has been suggested, however, that some Alectorioid species (*i.e. A. jubata*) may prefer acidic bark and that, as a result, moderate pollution stress from acid rain (which leads to bark acidification) may benefit these lichens, enabling them to colonize deciduous trees (Skye and Hallberg, 1969).

The oceanic boreal species *A. sarmentosa* is common in Newfoundland, but is largely confined to the coasts or the interior highlands (Ahti, 1983). *A. sarmentosa* has a pendent thallus, commonly 20-40 cm long (exceptionally to 80cm), greenish grey to bright golden yellow (Brodo and Hawksworth, 1977) and grows on a variety of trees, but especially conifers (*Picea* spp., *Tsuga heterophylla*, *Abies* spp., *Pseudotsuga menziesii* and *Pinus contorta*; Brodo and Hawsworth, 1977). The photobiont partner is believed to be *Trebouxia*, a chlorophyte alga (Brodo *et al.*, 2001). *A. sarmentosa* ranks with the terricolous species *Cladina mitis* and *Cladina rangiferina* as a preferred winter food for caribou (Brodo and

Hawksworth, 1977). Studies on the importance of lichen species in the diets of caribou populations across North America have estimated the summer diet to consist of 10-25% of lichens (Boertje, 1984; Gauthier *et al.*, 1989; Crête *et al.*, 1990), while the winter diet amounts to approximately 62% of lichens (Boertje, 1984). Rominger and Oldemeyer (1990) determined that during years of rapid snow accumulation, arboreal lichens such as *Alectoria* were the most important forage of caribou in autumn and winter. Thus, at least in part, the productivity of the lichen flora influences the carrying capacity of caribou in rangelands.

## 1.4 Biomonitoring with lichens

Lichens have become increasingly popular as natural monitors of pollution. This can be explained in part by the low cost of biomonitors compared to monitoring instruments, and the possibility of investigating almost limitless numbers of sites and of obtaining distribution patterns and comparative data (Galun and Ronen, 1988). The characteristics of an ideal biomonitor are defined by Puckett (1988) in the context of metal deposition studies, but can easily be extrapolated to other fields of interest. The organism:

1- must be capable of accumulating the substance of interest in measurable amounts,

2- must be available in terms of quantity and distribution, to avoid biased sampling,

3- must be available throughout the year,

4- should show differential uptake/accumulation related to exposure levels,

5- must not be subject to significant uptake of the substance of interest from other sources, when used in airborne contamination assessment,

6- must lend itself to repeated sampling,

7- must lend itself to cost-effective collection and analysis.

This section includes a brief look at the physiological characteristics that make lichens biomonitors of choice, an overview of different types of biomonitoring approaches, including transplant, and a survey of the known effects of pollutants on lichens, with an emphasis on $SO_2$.

### 1.4.1 Why does it work?

The first account of lichens being recognized as biomonitors dates from1866, when Nylander reported his finding that the lichen population in some Paris gardens was declining and attributed the decline to the presence of 'impurities' in the air: "*Les lichens donnent à leur manière la mesure de la salubrité de l'air et constituent une sorte d'hygiomètre très sensible*"[1].

Lichens, unlike higher plants, do not possess the ability to regulate the uptake of gaseous molecules and particulate matter from the atmosphere. They are devoid of such protective mechanisms as cuticles, wax layers or stomata (Richardson, 1988; Häffner *et al.*, 2001). The lack of protective mechanism and a root system result in the absorption of dissolved and particulate compounds anywhere on the lichen surface. Lichens, especially the fruticose filamentous type, have relatively high surface to volume ratios and have been

---

[1] The lichens provide in a way a measure of the salubrity of the air and constitute a kind of very sensitive hygiometre.

found to be more sensitive to atmospheric pollutants than corticolous lichens (Aguiar *et al.*, 1998; Häffner *et al.*, 2001). Early work in the field suggests that the relative resistance of lichens to air pollutants may be related to thallus morphology. Dässler and Ranft (1969) derived a tendency of increasing sensitivity from foliose, to fruticose to filamentous species, potentially related to proportional differences in the absorbing surface area (see section 1.4.3).

The ability of lichens to absorb compounds from the atmosphere well beyond their biological needs constitutes an advantage in the sense that it enables them to colonize a wide range of environments and to tolerate extreme natural climatic conditions (Galun and Ronen, 1988). Conversely, this mechanism increases the sensitivity of lichens to airborne pollutants.

### 1.4.2 Biomonitoring approaches

The use of lichens for biomonitoring purposes can be passive (*i.e.* qualitative) or active (*i.e.* quantitative). Passive monitoring entails the use of the distribution and composition of the lichen flora in a given area as an indicator of overall air quality (*e.g.* species richness is a function of air quality).

#### 1.4.2.1 Passive biomonitoring

This type of biomonitoring typically requires the prior classing of lichens into categories according to their relationship to air pollutants. One such classification scheme is given by Galun and Ronen (1988):

1. Sensitive species: include species on which pollutants have a rather detrimental effect and others with varying degrees of sensitivity. Eventually, all become deleted by air pollution.
2. Tolerant species: lichens species that are resistant to pollution and remain intact in their native habitat.
3. Replacement species: species that appear after the major part of the native lichen community has been destroyed by the effects of pollution.

In the early days of lichen biomonitoring, research often lead to the production of lichen distribution maps (*e.g.* Skye, 1968; Seitz, 1972; Sugiyama *et al.*, 1976). The studied site (usually a city) was divided into different zones: the lichen desert (main pollution centre; tree trunks bare of lichens), a struggle zone (around the pollution centre; tree trunks poorly colonized); and the normal zone (lichens thrive). More recent research typically includes additional variables in lichen distribution maps, such as frequency and coverage for example.

The index of atmospheric purity (I.A.P.) is a good example of a pollution index used in passive biomonitoring studies (LeBlanc and De Sloover, 1970) and is calculated as follows:

$$IAP = \frac{1}{10} \cdot \sum Q \cdot f$$

where n = number of species found at the station; Q = the ecological index of each species (the number of species found in the vicinity of the species studied at all the stations); and

f = the degree of frequency of cover for the species at the particular station. Species richness is proportional to the numerical value calculated with the IAP.

1.4.2.2 Active biomonitoring

Active monitoring involves the use of lichen thalli exposed to sites of interests or specific compounds or conditions where they function as integral indicators for the presence or absence of phytotoxic pollutants. Direct and indirect approaches may be used.

Using physiological responses of lichens to the presence of pollutants is one particularly prominent method of biomonitoring with lichens. In this approach, the physiological response of one or many lichen species to a specific pollutant, or a combination of pollutants, is investigated. Once such a response has been determined to result from exposure to toxins, it can be employed as an indicator. The investigation of physiological indicators has been performed for a number of toxins and combinations of toxins, from heavy metals (Garty *et al.*, 1992; Garty *et al.*, 1998b; Kauppi *et al.*, 1998; Takacs *et al.*, 1999; Pawlik-Skowronska *et al.*, 2001) to acidic deposition (Garty *et al.*, 1992; Kauppi *et al.*, 1998) and pollutants such as ozone (Menzel, 1976; Sigal and Nash, 1983; Eversman and Sigal, 1987), fluorides (Leblanc *et al.*, 1972; Perkins and Millar, 1987a; 1987b) and sulphur dioxide (see section 1.4.3).

Because it may be difficult to measure the impact of a particular compound on lichens in a natural setting, many of the findings which relate the effects of toxins on lichen physiology have been made using controlled experimentation techniques. Controlled

exposure to pollutants may be achieved through such methods as the use of fumigation chambers (Fields and St. Clair, 1984; Eversman and Sigal, 1987; Balaguer and Manrique, 1991; Balaguer *et al.*, 1997; Deltoro *et al.*, 1999;; Kong *et al.*, 1999) or the soaking of lichen thalli in solutions containing specific compounds or pollutants (Garty *et al.*, 1992; Kauppi *et al.*, 1998). Fumigation has also been performed in the field (Moser *et al.*, 1983).

Biomonitoring studies are usually conducted in proximity to a site of interest, for example in urban areas or near the location of a specific industrial installation (smelter, oil refinery, etc.). To monitor atmospheric pollution, lichens may be sampled at the site(s) of interest and compared to other lichens of the same species growing in areas that are considered to be "unpolluted". Damage to the lichens is assessed by evaluating certain physiological parameters in the two groups, and then comparing them.

Lichens can also be used as monitors of atmospheric pollutant deposition for such compounds as metals, radioactive elements, pesticides, sulphur, etc. Because the uptake mechanism in lichens is largely passive, prolonged exposure to a pollutant typically increases concentrations (sulphur: Gilbert, 1965; Leblanc and Rao, 1973; Kauppi and Halonen, 1992; Häffner *et al.*, 2001; Wiseman and Wadleigh, 2002, metals and trace elements: Kauppi and Halonen, 1992; Tuba and Csintalan, 1993; Sloof, 1995). The content of a particular substance in a lichen can thus be measured and related to patterns of deposition. For example, studies have shown that heavy metal concentrations in lichens can be correlated with bulk deposition rates of heavy metals (Pilegaard, 1979; Sloof, 1995). Another example is an epidemiological survey of the incidence of lung cancer in an area

near a steel foundry in Scotland conducted by Gailey *et al.* (1985), in which the main areas of metal deposition were identified by measuring the metal content in lichens after prolonged exposure to the foundry emissions.

1.4.2.2.1 Transplant studies

A method of conducting biomonitoring studies with lichen involves the 'transplanting' of lichen thalli. Transplanting consists in the relocation of lichen thalli originating from an area relatively untouched by toxic emissions to a site of interest (or the opposite, in rare cases). The method of transplant used is largely dependent on the morphological type of the lichen species used in the study. In the case of crustose and foliose lichens, the substrate is usually removed with the lichen thallus to avoid damaging it. This may be rather difficult when the substrate is rock, but relatively simple in the case of bark. Brodo (1961) was the first to use a transplant method for foliose lichens. Bark cores supporting lichens were removed from oak trees and affixed to host trees with grafting wax. Bark 'plugs' have also been inserted in wooden exposure plates when no suitable host trees could be found (Schonbeck and van Hut, 1971). Lichens from unpolluted regions can be transplanted to a particular site of interest, or along a pollution gradient (Tarhanen *et al.*, 2000; Häffner *et al.*, 2001). Häffner *et al.* (2001) exposed thalli of four lichen species at ten sites along an $SO_2$ gradient in Germany and the Czech Republic for 4 months. At 4-weeks intervals, samples were removed and tested for element and anion content, pigment content (chlorophylls, phaeophytins, carotenoids), respiration and photosynthesis rates. Thallus

bleaching, which indicates chlorophyll loss, was found to occur in all species, in some sites as early as 4 weeks after the onset of exposure. All species suffered chlorophyll loss, membrane leakage, and decreased respiration and photosynthesis rates. Total sulphur concentrations were found to reflect mean sulphur immissions at the sites and increased over the course of the experiment. Fruticose species were found to be more sensitive to $SO_2$ stress than other growth forms.

Crustose lichens are particularly useful in metal accumulation studies because they are, as a general rule, less sensitive than fruticose lichens. They can also be used for biomonitoring in cases where fruticose lichens are rare, (Purvis *et al.*, 2000), such as in arid regions. Fruticose lichens are, however, the preferred type of lichen in biomonitoring studies. This is due in part to the fact that these are easier to handle (the thalli are usually much larger and rarely attached securely, if at all, to the substrate), are generally more sensitive to pollutants, and also because of the greater incidence of purely epiphytic lichen in this particular morphological type. Fruticose lichens may be removed from the substrate and put into mesh bags, which are then attached to a pole or tree (Gonzalez *et al.*, 1998; Gonzalez and Pignata, 2000; Carreras and Pignata, 2001); or the substrate (twig or branch) can be removed along with it and reattached somewhere else. This last method has the advantage of reducing the disturbance to the lichen and has been used in a large number of studies, including this one.

One consideration in studies that rely on the relocation, or transplant, of lichens to a different location is whether or not the act of relocation itself may account for some or all

of the observed response. In the case where fruticose epiphytic lichens are not removed from their substrate (usually twigs or branches), one would assume that the transplantation is unlikely to have an effect. Nevertheless, some have seen fit to verify this hypothesis.

Garty *et al.* (1993) found no significant differences in ethylene production at a control site between *Ramalina duriaei* (a fruticose, epiphytic lichen) thalli retrieved *in situ* and thalli resuspended in the same area. Zambrano *et al.* (1999) and Zambrano and Nash (2000) also tested the hypothesis that the transplant itself could modify lichen response, but found no significant transplant affect for all variables tested (net photosynthesis, chlorophyll content, total carotenes in both studies) for lichens relocated in same site and undisturbed lichens in the same area.

It is possible to bypass the investigation of the transplant response factor by transplanting a portion of the lichens to a study site, while re-transplanting the remainder of the lichens on the tree of origin. The resuspended lichens therefore serve as a basis for comparison, eliminating the need to consider the effect of the relocation itself, since all lichens are submitted to the same manipulations. This is by far the preferred strategy (Garty *et al.*, 1993; 1997a; 1997b; 1997c; 1998a; 1998b; 2001).

### 1.4.3 Lichens and atmospheric pollutants

Fumigation studies are especially useful for investigating the physiological responses of lichens to specific air pollutants and have been performed for such pollutants as sulphur dioxide, hydrogen fluoride, ozone, nitrogen dioxide and peroxyacetyl nitrate. Fields (1988)

describes the order of sensitivity of lichen physiological processes in fumigation studies as follows: $N_2$ fixation (in cyanolichens) > K+ efflux/total electrolyte leakage > photosynthesis, respiration > pigment status.

1.4.3.1 Sulphur dioxide

$SO_2$ is a major component of urban and industrial atmospheres and a wealth of studies have shown that it can be extremely deleterious to lichens, to the extent of eradicating sensitive species. Sulphur dioxide is a very soluble gas and is thus easily dissolved in rain water or moisture present within the thallus. At low pH, $SO_2$ combines with water to form sulphurous acid, at which point it can be oxidized by $O_2$ to $H_2SO_4$ (Nielsen, 1972). Sulphurous and sulphuric acid possess oxidizing power, but relatively little compared to the compounds that can form at higher pH. In such conditions, $SO_2$ forms the sulphite or bisulphite ions, which then combines with water before being oxidized to the sulphate ion as part of the lichen resistance mechanism (Richardson, 1992; Kong *et al.*, 1999). Of particular interest are the free radicals that can be produced as a result of this reaction sequence, notably the superoxide radical ($O_2^-$) (Tan and Liu, 1981), as these are believed to influence every aspect of lichen physiology. $SO_2$ is thought to be metabolized within the photobiont cells of the thallus (Lange *et al.*, 1989).

Sulphur dioxide affects biological systems in two ways: through acidification and through oxidation. In the case of lichens, oxididation is considered to be the dominant effect. The phaeophytinization of chlorophyll is a result of acidification and can be used as a

physiological indicator or $SO_2$-induced damage (Balaguer *et al.*, 1997; Gonzales and Pignata, 1997; 2000; Kong *et al.*, 1999). Physiological responses to the oxidating capacity of $SO_2$ include, but are not restricted to: pigment bleaching or degradation (Henriksson and Pearson, 1981; González *et al.*, 1996; Kong *et al.*, 1999), the production of peroxidation products, such as hydroperoxy-conjugated dienes and maliondialdehyde (Gonzales and Pignata, 1997; Gonzales *et al.*, 1998; Carreras and Pignata, 2001), membrane injury and subsequent leakage of electrolytes (Puckett *et al.*, 1974; Tomassini *et al.*, 1977; Pearson and Henriksson, 1981; Pearson and Rodgers, 1982), peroxidation of membrane lipids (Gonzales and Pignata, 1994; Levin and Pignata, 1995). Other responses of lichens to $SO_2$ oxidative stress are: the production of stress-ethylene (Epstein *et al.*, 1986), changes in the level of endogenous auxin (Epstein *et al.*, 1986) decrease in photosynthetic and respiration capacity, eventually leading to complete photosynthetic breakdown (Moser *et al.*, 1983; Häffner *et al.*, 2001),

Visible effects of $SO_2$ stress include bleaching of the thallus or loss of pigmentation (Moser *et al.*, 1983; Häffner *et al.*, 2001). Häffner *et al.* (2001) studied the physiological responses of four lichen species in a transplant experiment along an $SO_2$ gradient. All lichens exposed underwent bleaching and discolouration of the thallus with time, indicating chlorophyll destruction. Sites of facilitated gas exchange such as apical regions, soralia and pseudocyphelles were preferentially bleached following exposure to $SO_2$ gas.

Sulphur can be accumulated in lichens as a result of the metabolizing of sulphur dioxide or the uptake of sulphate from acidic deposition or wind-blown sea spray. Many studies have suggested a positive correlation between sulphur concentration in lichens and

the amount of $SO_2$ in the air (Gilbert, 1973; Puckett and Fineman, 1980; Hopp and Kappen, 1981; Richardson and Nieboer, 1983), suggesting that sulphur accumulation in lichens provides a reasonable estimate of $SO_2$ concentration in the atmosphere (Garty *et al.*, 1977; 1985; Levin and Pignata, 1995). Sulphur content has also been used to assess pollution damage to lichens in association with specific emission sources (Richardson and Nieboer, 1981; Showman and Long, 1992; Gonzales and Pignata, 1994; Garty *et al.*, 1996) and has been correlated in sensitive species to the impact on physiological parameters (Malhotra and Khan, 1983; Garty *et al.*, 1985; Häffner *et al.*, 2001). Studies have shown that lichens located closer to urban centres tend to have greater sulphur concentration. For example, Nieboer and Richardson (1981) found that *Cladonia mitis* thalli collected within a ten-mile radius of a nickel smelter in Sudbury, Ontario, Canada, had a sulphur content that was more than twice the local background level. In a study that aimed at tracing the sources of sulphur in Newfoundland, Canada, using stable isotopes of sulphur, Wadleigh and Blake (1999) found the highest sulphur contents in lichens associated with known industrial point sources.

### 1.4.4 Lichens and stable isotopes

Not many studies have investigated stable isotopes in lichens. Carbon isotope discrimination techniques have been used for a few lichen species, in an attempt to characterize the photobiont associations (Maguás *et al.*, 1993). In the lichens that were studied, the least discrimination occurred in lichens with a single photobiont (discrimination was least in associations with a cyanobiont), whereas lichens with cyanobacteria in

cephalopodia showed the highest discrimination. Maguás and Brugnoli (1996) also used carbon isotope discrimination techniques to investigate spatial variation in photosynthetic uptake and carbon discrimination. Their results suggested that spatial variations in carbon discrimination across the thalli of five lichen species (*Lasallia postulata*, *Lobaria amplissima*, *L. pulmonaria*, *L. scrobiculata* and *Peltigera canina*) were related to thallus age: carbon discrimination was greatest in marginal regions of the thallus (tips) compared to the central and basal regions. Teeri (1981) investigated the stable carbon isotope composition of six lichen species and determined that all species sampled exhibited carbon isotopic compositions consistent with the RUBP carboxylase photosynthetic pathway as a main method of $CO_2$ uptake, with some small influence from another pathway, possibly PEP carboxylase (mean $\delta^{13}C$: -23.7‰).

Krouse (1977) measured the sulphur isotopic composition of different materials (air, lichens, soil, pine needles) and found that the fruticose lichens of the genus *Usnea* had isotopic compositions that coincided closely with the air, which suggested that uptake of sulphur from the atmosphere by lichens occurs via direct pathways. Wiseman and Wadleigh (2002), investigated the response of transplanted fruticose lichens of the species *Alectoria sarmentosa* to changes in levels of sulphur atmospheric pollution by measuring sulphur isotopic composition and concentration. The lichens were transplanted from an area where the main source of sulphur is sea spray to an area dominated by anthropogenic sources, and monitored for one year. The sulphur isotopic composition was found to decrease semi-linearly, while the concentration increased semi-linearly. A period of 18 months would have

been necessary for the lichens to reach local sulphur conditions.

Table 1.1: Atmospheric sulphur species; formulas, valences, sources, sinks and residence times[a]

| Species | Formula | Valence | Sources | |
|---|---|---|---|---|
| | | | *Natural* | |
| Dimethyl sulphide (DMS) | $CH_3SCH_3$ | -2 | *Oceanic (99%; 16TgSyr$^{-1}$)*[b]<br>- Phytoplankton (breakdown of DMSP)<br>- Coastal seaweed beds<br><br>*Terrestrial (1%)*[b]<br>- Organic decomposition | - - - |
| Carbonyl Sulfide | OCS | -2 | *Oceanic (0.3 TgS yr-1)*[b]<br>- Vegegation<br><br>*Terrestrial (0.3 TgS yr-1)*[b]<br>- Vegetation<br>- Soils | - Biomass |
| Hydrogen sulfide | $H_2S$ | -2 | - Volcanoes (0.1 TgS yr$^{-1}$)[b]<br>- Terrestrial plants and soils<br>- Oceans<br>- Microbial sulphate reduction | - Biomass<br>- Industria |
| Carbon disulfide | $CS_2$ | -2 | - Vegetation<br>- Soils | - Chemica industry) |
| Sulphur dioxide | $SO_2$ | +4 | - Volcanoes (10%)[c]<br>- Vegetation | - Fossil fu<br>- Coal (5<br>- Biomass |
| Methane sulfonic acid (MSA) | $CH_3SO_3H$ | +4 | - End product of DMS oxidation | - - - |
| Sulphate | $SO_4^{2-}$ | +6 | - Sea spray<br>- Oxidation of $SO_2$<br>- Aeolian soil weathering (0.19 - 19 TgS yr$^{-1}$)[d] | - Oxidatio |

a: List is not exhaustive. Intermediate products are not mentioned. Major sources and sinks only are identified.
b: Brasseur, 1999
c: Bates *et al.*, 1992
d: Aneja, 1990
e: all processes within clouds that result in removal from the atmosphere
f: all processes of removal by precipitation below clouds
g: Nielsen, 1972
h: Kurylo, 1978
i: Rodhe, 1978
j: Cox and Sheppard, 1980

d residence times[a]

| Sources | Sinks | Residence time |
|---|---|---|
| *Anthropogenic* | | |
| - - - | Gas-phase<br>- Reaction with OH radical<br>- Reaction with $NO_3$ radical (in coastal zones, at night)<br>- dissolution in aqueous particles | < 1 day[i] |
| )MSP) | Seawater<br>- Biological/chemical consumption | |
| - Biomass burning (12%)[b] | Tropospheric<br>- Uptake by soils<br>- Uptake by vegetation<br>- Hydrolysis by natural waters<br>- Oxidation of $CS_2$<br>- UV-initiated breakdown of dissolved organic matter | Global<br>- 500 days[h] |
| | Stratospheric<br>- Photolysis by UV radiation<br>- $SO_2$ and sulfate particle formation | |
| - Biomass burning<br>- Industrial activities | - Reaction with OH radical | 1 day[i] |
| - Chemical processing (especially cellulose industry) | - Reaction with OH radical (to form OCS) | 70 days[j] |
| - Fossil fuel combustion<br>- Coal (53%)[b], Oil (28%)[b]<br>- Biomass burning (1.4 - 2.9 TgS $yr^{-1}$, 2%)[c] | - Dissolution in aqueous droplets (to form $H_2SO_4$), followed by liquid-phase oxidation<br>- Reaction with OH radical | 1 day[i] |
| - - - | - Condensation in the liquid phase<br>- Deposition onto existing particles | N.A. |
| - Oxidation of anthropogenic $SO_2$ | - Rainout[e]<br>- Washout[f]<br>- Dry removal<br>- Dissolution into oceans?[g] | Over land<br>- several days[g]<br>Cloud level<br>- 1 week[g] |
| 19 TgS $yr^{-1}$)[d] | | |

re identified.

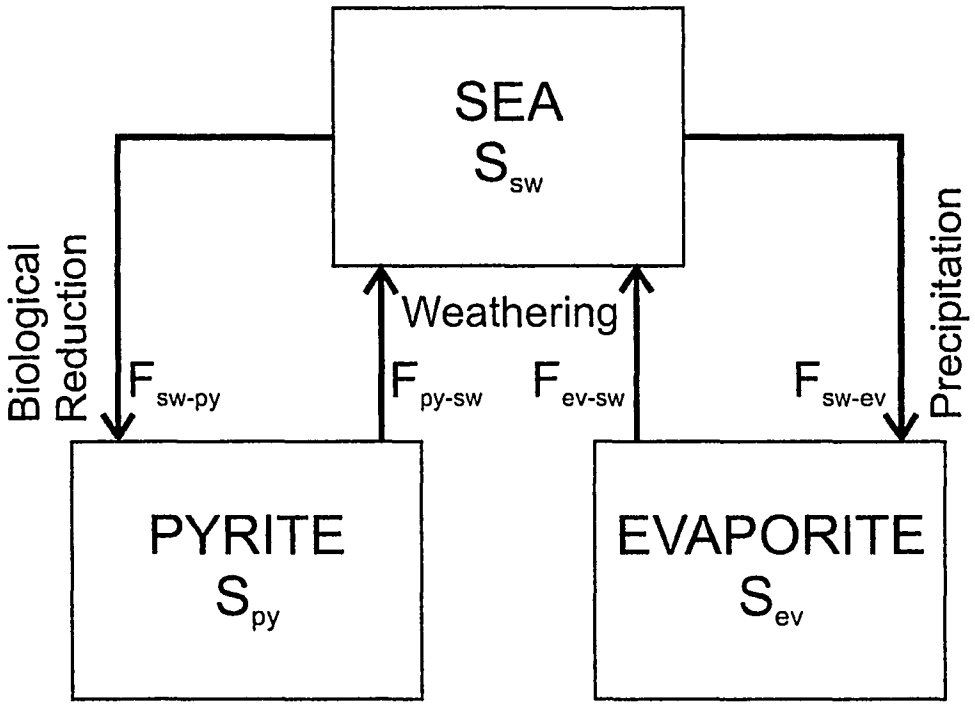



Figure 1.1: Simplified box model of the global sulphur cycle (modified from Holser *et al.*, 1989)

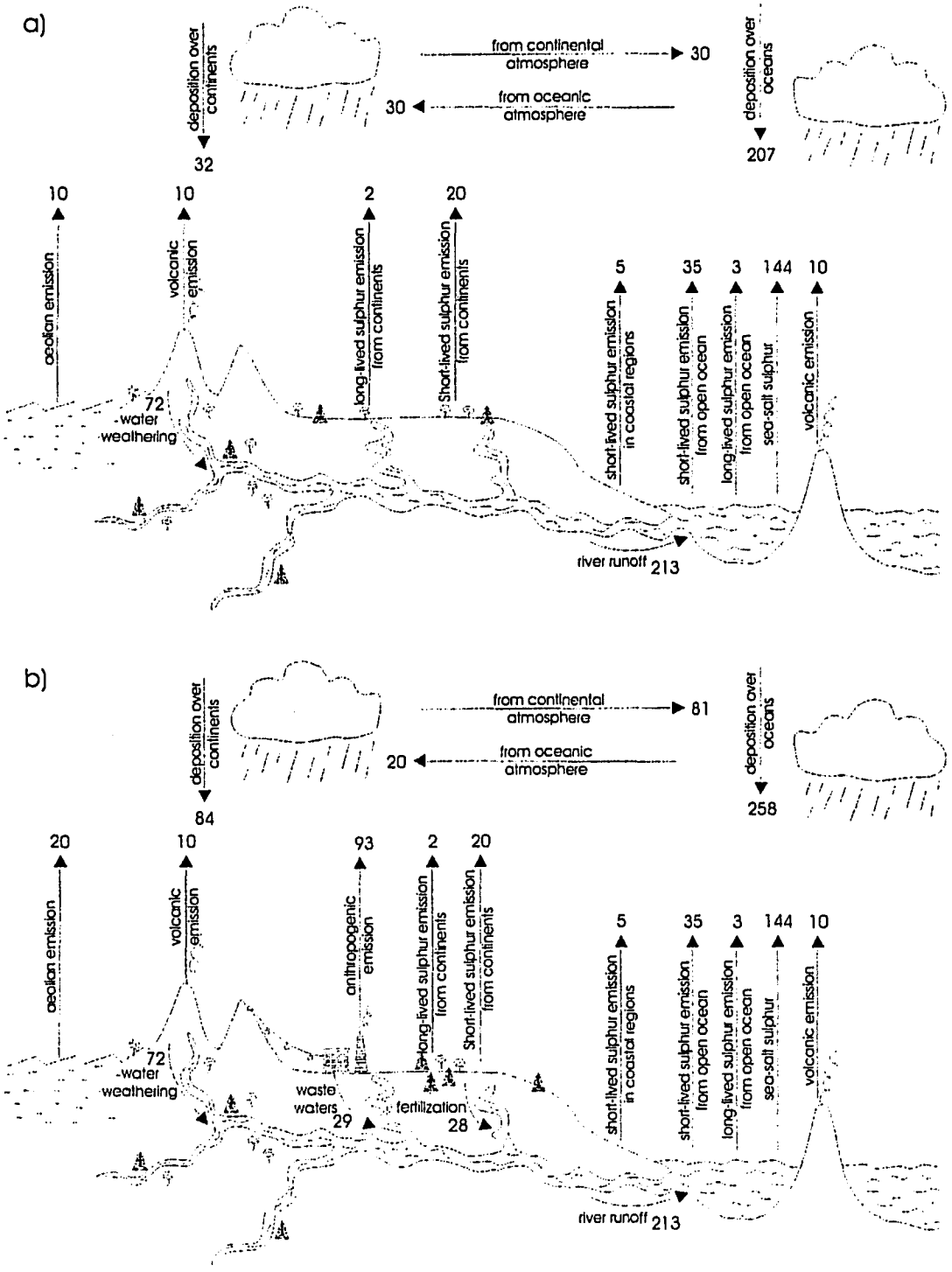



Figure 1.2: a) The primitive sulphur cycle. b) The sulphur cycle today. Fluxes are in Tg S $a^{-1}$ (from Brimblecombe, *et al.*, 1989)

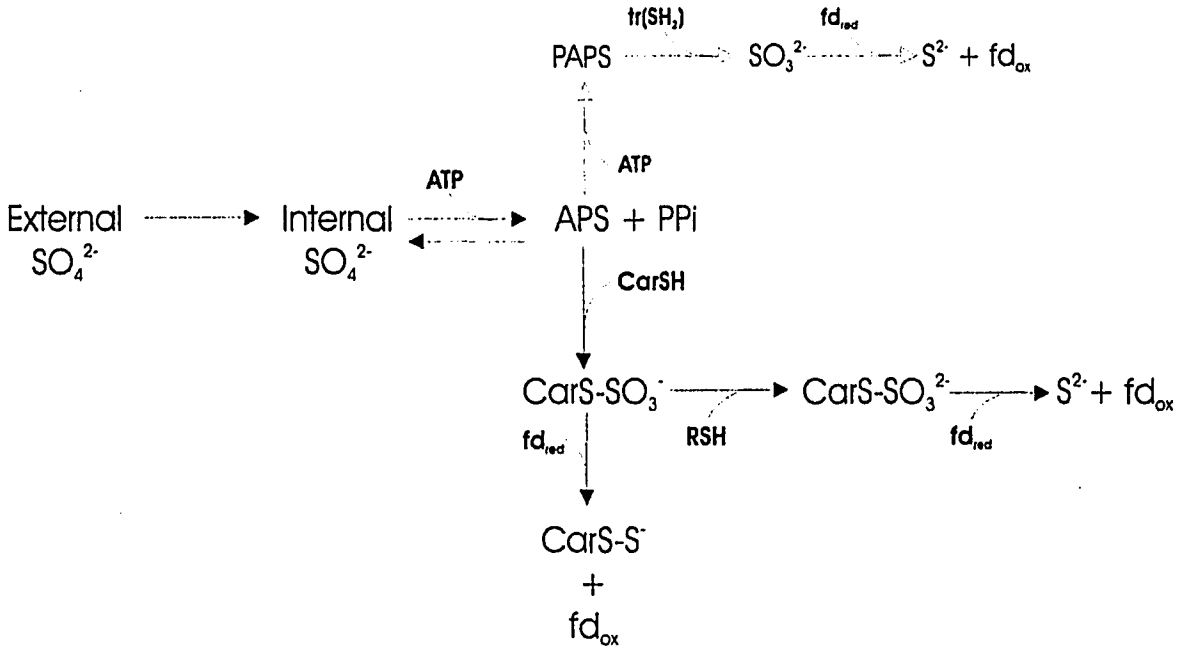



Figure 1.3: Reaction sequence for the assimilatory pathway of sulphate reduction. Black arrows: major pathway, used by eukaryotic algae and higher plants. White arrows: secondary pathway, used by bacteria, some cyanobacteria (modified from Brunold, 1990).

APS = adenosine-5'-phosphosulphate; ATP = adenosine triphosphate; CarSH = unidentified carrier molecule; CarS-S$^-$ = carrier-bound sulphide; CarS-SO$_3^-$ = carrier-bound sulphite; $fd_{red}$ = reduced ferredoxin; $fd_{ox}$ = oxidized ferredoxin; PAPS = adenosine 3'-phosphate 5'-phosphosulphate; PPi = inorganic pyrophosphate; RSH = a thiol molecule; tr(SH$_2$) = reduced thioredoxin.

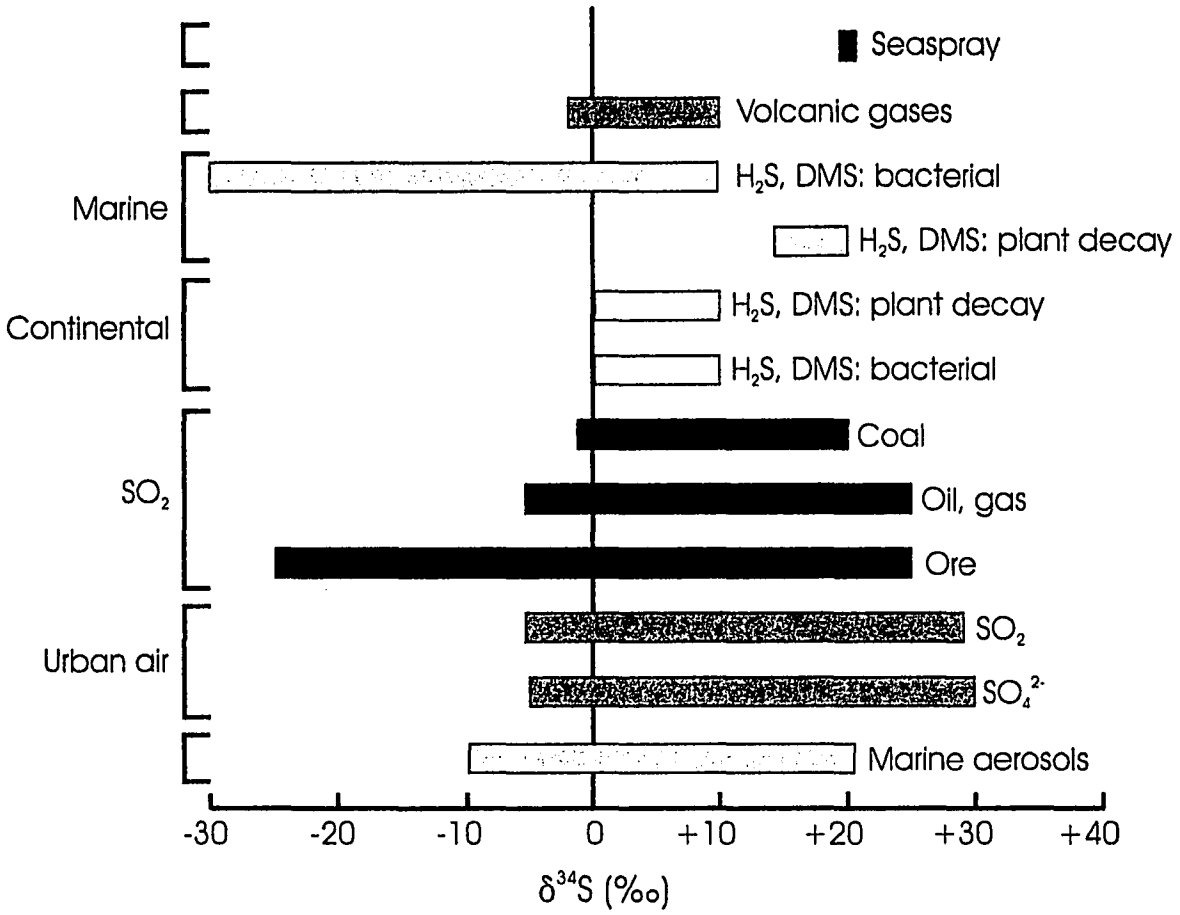



Figure 1.4: Sulphur isotopic composition distribution for the source materials of atmospheric sulphur (Modified from Newman *et al.*, 1991)

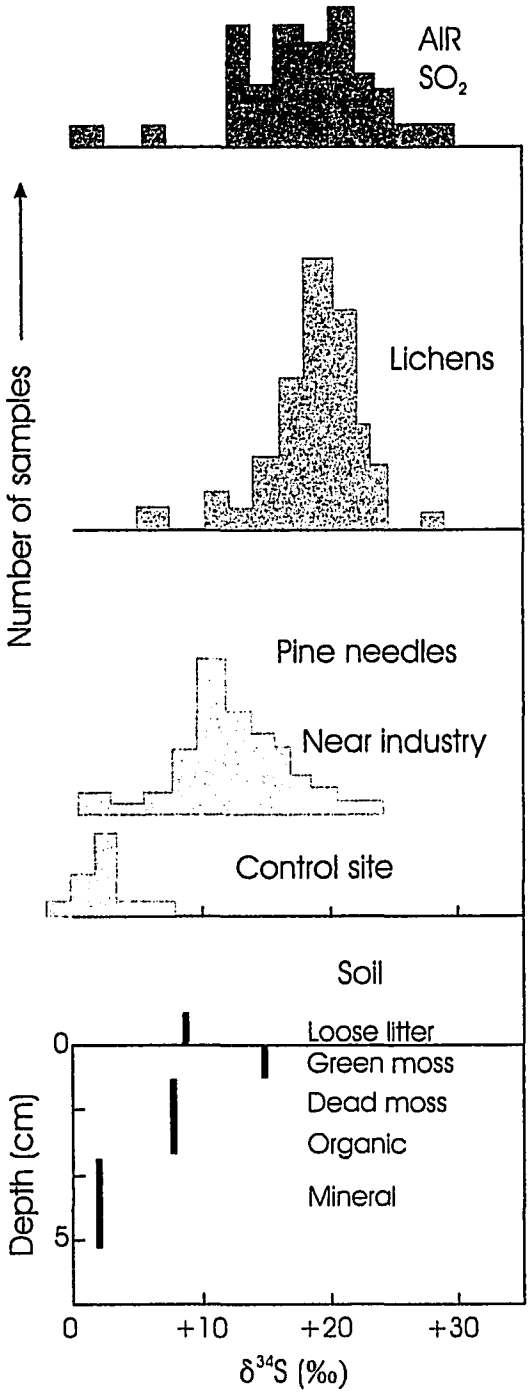



Figure 1.5: $\delta^{34}S$ for atmospheric $SO_2$, vegetation and soil in Ram River, Alberta (1971-1972) (modified from Krouse *et al.*, 1991)

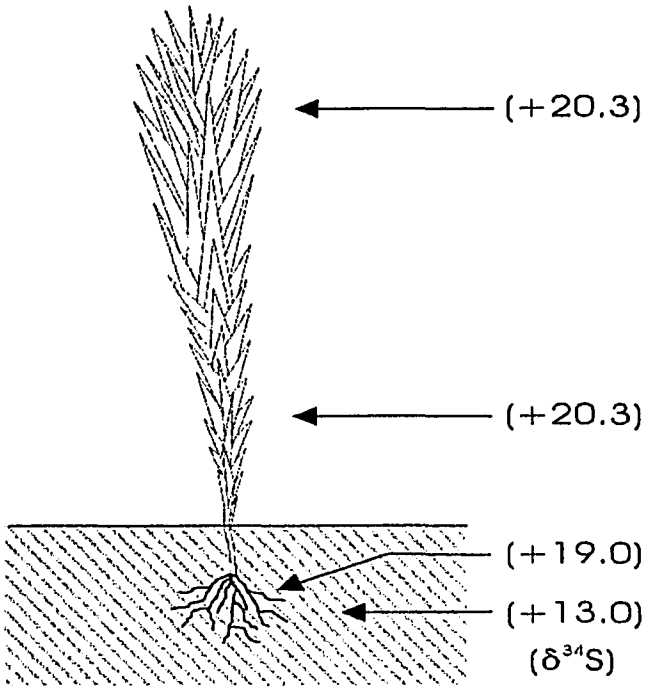



Figure 1.6: $\delta^{34}S$ for different portions of the moss *Polytrichum juniperinum* (modified from Krouse *et al.*, 1984)

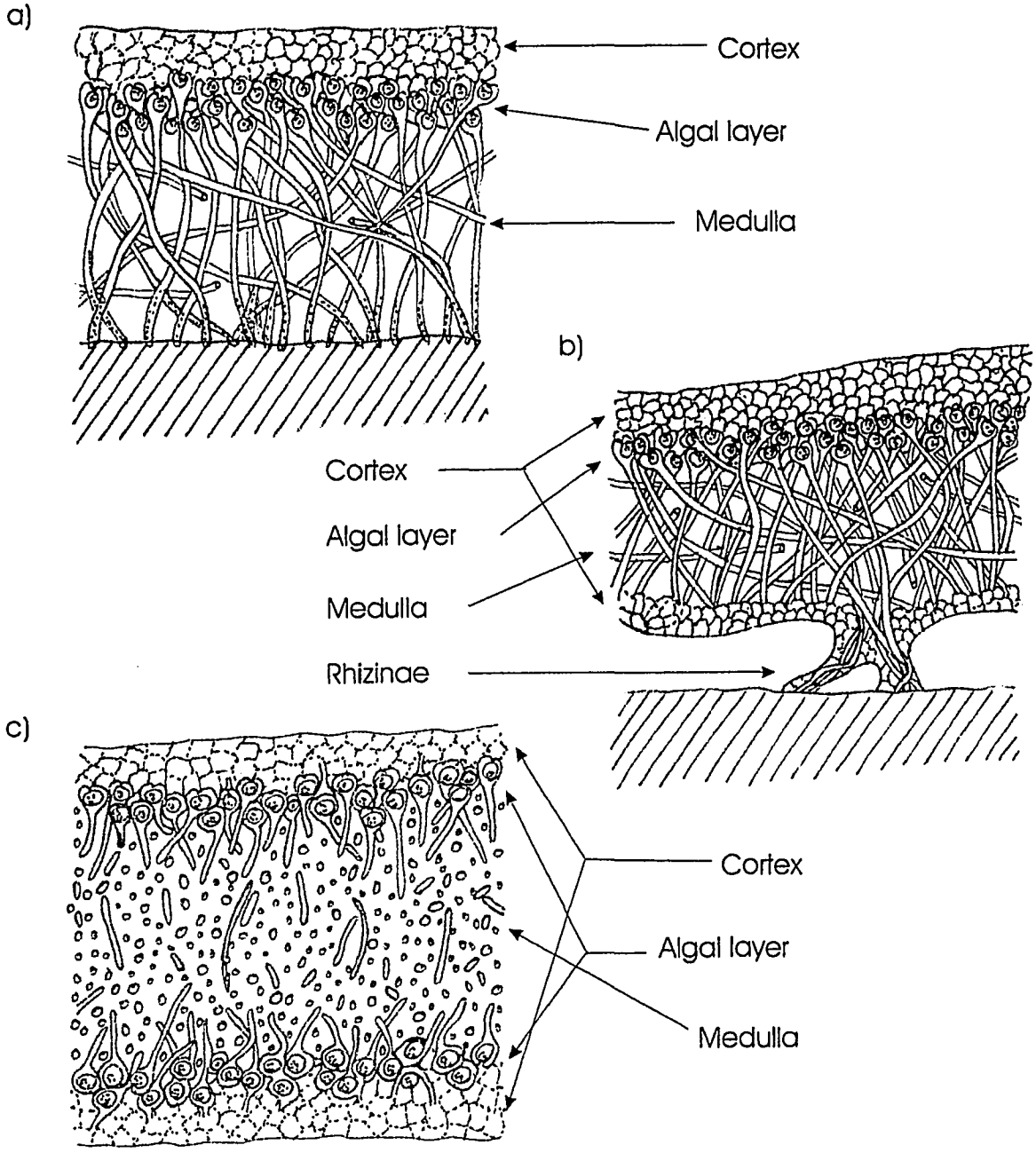



Figure 1.7: Vertical sections of the crustose (a) and foliose (b) growth forms and horizontal section of the fruticose (c) growth form (modified from Ahmadjian, 1967).

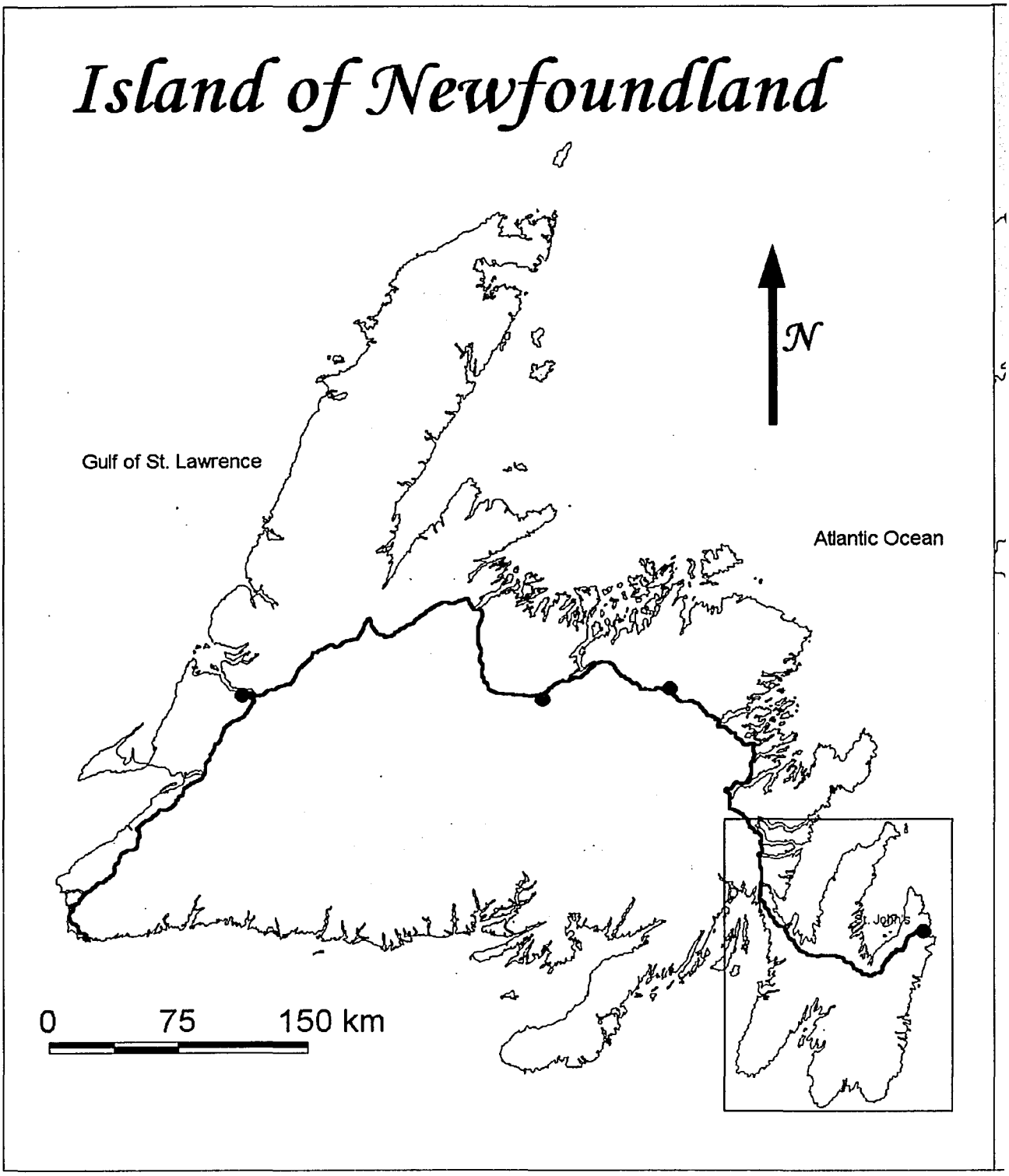
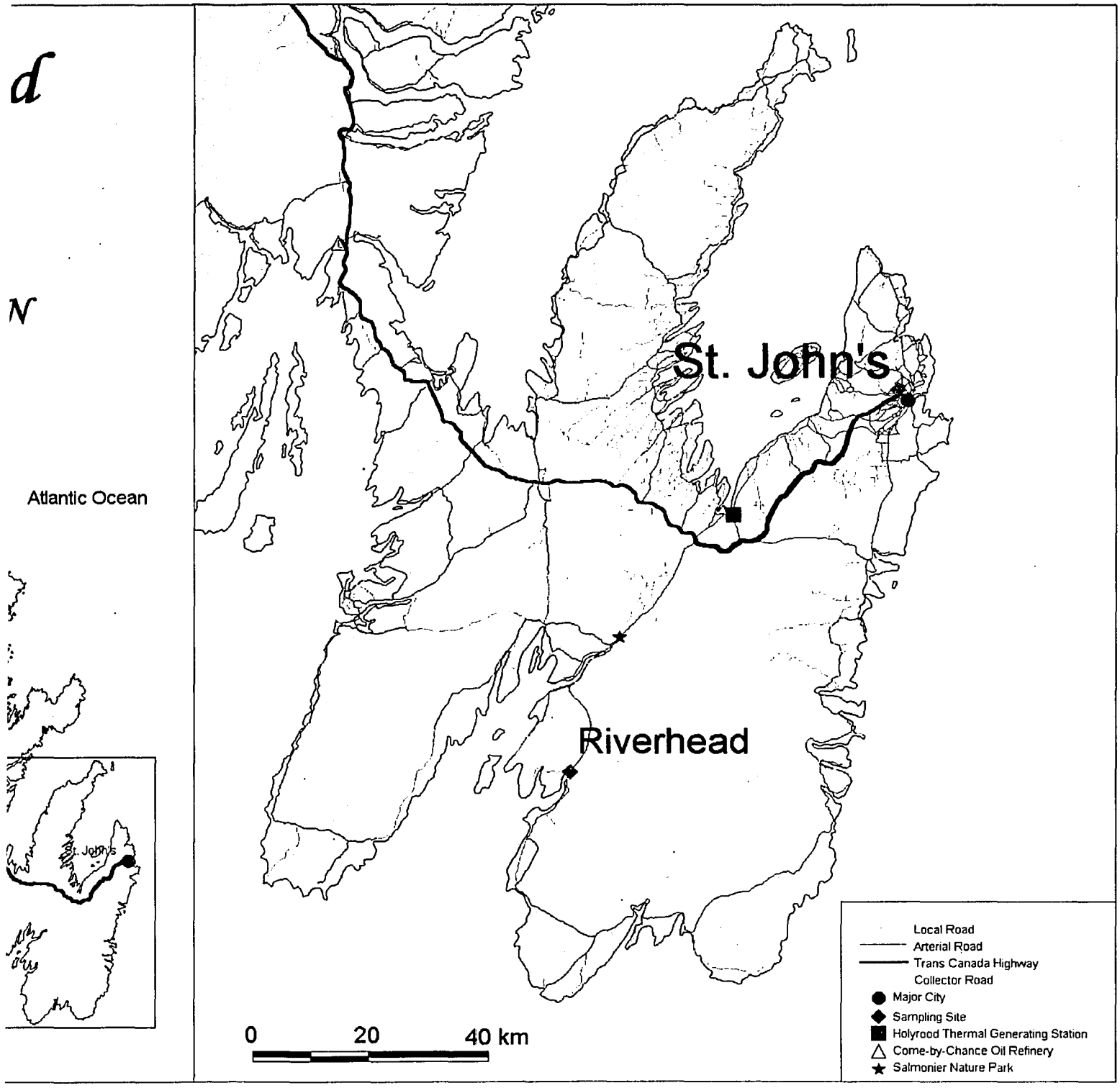



Figure 1.8: Map of the island of Newfoundland and the Avalon Peninsula (inset).


St. John's
Riverhead
Atlantic Ocean
0
20
40 km
Local Road
Arterial Road
Trans Canada Highway
Collector Road
Major City
Sampling Site
Holyrood Thermal Generating Station
Come-by-Chance Oil Refinery
Salmonier Nature Park

set).

# Chapter 2: Materials and Methods

## 2.1 Choice of biomonitor

The choice of *A. sarmentosa* as a biomonitor for this study stems from a number of considerations. Firstly, *A. sarmentosa* is abundant in Newfoundland, especially along the coasts (Ahti, 1983). Secondly, it is relatively easy to identify, as only a few other lichens (*e.g.*, *Bryoria* spp.) in the Newfoundland region resemble it. Thirdly, the mass of an *A. sarmentosa* thallus is sufficiently large for sulphur isotope analysis, if one considers that approximately 9μg of sulphur in the form of $SO_2$ in each sample processed is necessary to ensure reasonable accuracy and confidence in the isotope ratio measurements using current technology (Yun, 2000). Fourthly, *A. sarmentosa* is a pendulous lichen easily separated from its sulbstrate. Lastly, *A. sarmentosa* is an epiphyte and therefore not likely to be influenced by its substrate, thus eliminating the need to consider nutrient uptake from the substrate.

## 2.2 Study area

### 2.2.1 Sulphur sources in Newfoundland

Point sources of sulphur in the atmosphere of Newfoundland include the Come-by-Chance oil refinery, several teepee-type incinerators, and some oil-fired power generating stations (Figure 1.8). The Come-by-Chance oil refinery (North Atlantic Refining), located approximately 100 km from St. John's, in southeastern Newfoundland, Canada, is the largest single point source of $SO_2$ on the island of Newfoundland. Prior to 2000, sulphur emissions from the refinery amounted to 35,000 t $SO_2$ per year on average, but process modifications

in 2000/2001 reduced emissions to approximately 15,000 t of $SO_2$ per year. The isotopic composition of the sulphur originating from the burning of Bunker C fuel for power generation has not been measured directly, but is probably similar to the average for eastern North America ($\delta^{34}S \sim +5$ ‰) and $\delta^{34}S$ values measured in epiphytic lichens from the area (Wadleigh and Blake, 1999). Fuel samples from the refinery were analysed for $\delta^{34}S$; values ranged from −2.98 to 0 ‰ (Ennis, 1999).

The oil-fired Holyrood Generating Station is located approximately 2.5 km from Seal Cove, a small municipality (pop. < 500) 35 km northwest of St. John's, and operates mainly during the winter months. Emissions are estimated at 15 000 tonnes $SO_2$ annually.

A small oil-fired power plant is also located on the Memorial University of Newfoundland St. John's campus and is operated year-round to provide heat and emergency power to adjoining buildings. Sulphur emissions are less than 1000 tonnes $SO_2$ per year.

Diffuse sources of sulphur on the Avalon Peninsula include motor vehicles, home heating (mainly oil-based), and sea spray sulphates.

### 2.2.2 Sampling sites

The prime requirement for the choice of the two sampling locations was the existence of a large disparity in their respective sulphur isotopic compositions and concentrations. This was to ensure that between-site differences in measurements exceeded natural variability within the lichen population. The disparity was assessed using results from a previous study by Wadleigh and Blake (1999) in which the sulphur isotopic

composition was measured in lichens of the *Alectoria sarmentosa* species throughout the island of Newfoundland, and through preliminary sampling performed at the Riverhead site on 27 October 2001, which indicated a dominant coastal influence ($\delta^{34}S = 17.8 \pm 1.7$ ‰; $n = 9$).

A second requirement for the choice of sites was accessibility, as regular visits were required over an 18-month period. Road closure due to snow accumulation and high winds is frequent on the Avalon Peninsula, Newfoundland.

A last prerequisite was the absence of significant long range transport of pollutants from mainland Canada on the Avalon Peninsula. This has been verified by Wadleigh and Blake (1999) in a lichen biomonitoring study. Measured sulphur isotopic composition will therefore reflect mostly local sources.

#### 2.2.2.1 MUN Botanical Garden, St. John's

The MUN Botanical Garden (BG) is located in St. John's, Newfoundland, on the Avalon Peninsula (Figure 1.8: inset). This site was chosen to represent an urban/polluted location. Atmospheric sulphur in the area is predominantly of anthropogenic origin (Jamieson and Wadleigh, 1999; Jamieson and Wadleigh, 2000). Sources include the burning of fossil fuels for transportation, heating and electricity generation, and incinerators. The sulphur isotopic composition, as measured in precipitation and lichens, is approximately + 6 ‰ (Wadleigh and Blake, 1999; initial sampling).

The site is in a natural stand typical of a mixedwood boreal forest, with the majority of trees of coniferous species, such as *Picea spp.* and *Abies balsamea.* Deciduous species present at the site include *Betula papyrifera*, *Betula populifolia*, *Salix bebbiana* and *Populus tremuloides.* Undergrowth vegetation is dominated by *Cornus canadensis* and various ericaceous species (*Kalmia polifolia, Gaultheria hispidula*).

2.2.2.2 Riverhead (St. Mary's Bay)

The community of Riverhead, on the Avalon Peninsula, is located 90 km south-west of St. John's and represents a marine/unpolluted location. The sulphur is believed to originate chiefly from sea spray sulphates, as indicated by sulphur isotopic compositions in vegetation approaching +20‰ (Wadleigh and Blake, 1999; this study). The vegetation is typical of a lower foothill forest and is dominated by a single tree species, *Picea glauca.* The forest floor is poorly drained and is covered by various moss species, including sphagnum moss. Undergrowth vegetation is dominated by ericaceous species (*Ledum groenlandicum*, *Kalmia polifolia*, *Oxycoccus microcarpus*). Other species of importance include *Eriophorum* spp. and *Cornus canadensis.*

2.3 Experimental design

The experimental design was determined and tested prior to the start of the experiment using data from a similar study by Wiseman and Wadleigh (2002) and general linear models (GLM). Two types of design were considered for this experiment: a

randomized complete block design and a completely randomized design. The randomized complete block design would have permitted us to account for non-homogeneity within trees, or for the possibility that the tree on which a lichen is transplanted can act as a variable in itself. This scheme would then have permitted us to follow each group of trees through time, because if isotopic ratios vary from tree to tree, then a time-dependence of the isotopic ratio from randomly selected samples alone cannot be expected. In order to test this possibility of a "tree" factor, 30 lichens from 5 trees were sampled at Riverhead in October 2001 and analysed for their sulphur isotopic composition and concentration (Table 2.1). The *p*-values obtained with a one-way ANOVA where 0.049 for $\delta^{34}S$ and 0.109 for [S], indicating that at a confidence level of 90%, the tree on which lichen is transplanted does not have a significant effect on its response to changes in atmospheric sulphur. A confidence level of 90% was used in this case because of the small sample sizes. Although randomized complete block designs make better use of information, completely randomized designs allow for simpler sampling strategies and statistical analyses. In view of this, a completely randomized design was used in this study.

Three components were included in the experimental design: a reciprocal transplant effect (T: intersite relocation), a local transplant effect (TC: intrasite relocation), and *in situ* variation (C: undisturbed lichens, or reference). The intersite relocation treatment (T) is the basis of the experiment and was expected to provide information on the response of lichens to changes in the concentration and isotopic composition of atmospheric sulphur. The local transplant or intrasite relocation (TC) component was added to determine whether the act

of re-suspending lichen material alters lichen response. The reference element (C) served the purpose of assessing the natural seasonal variability in the concentration and isotopic composition of atmospheric sulphur, while at the same time providing a baseline level with which to compare the transplanted lichen data.

## 2.4 Experiment setup

The experiment was set up at both sites between 10 and 13 November 2001. Thirty trees which met the following conditions were haphazardly selected at each site and numbered: i) the tree was alive; ii) the tree was of a coniferous species (such as *Abies balsamea* or *Picea abies*); and iii) the tree was supporting healthy individuals of the *Alectoria sarmentosa* species (this served as proxy information that the trees provided a suitable habitat for the lichens). Each tree was identified with a tag bearing a number from 1 to 30 and the location (RH for Riverhead, BG for MUN Botanical Garden).

At each site, branches at heights no greater than 2.5 m were taken from each of the 30 trees and stored in clear plastic bags until relocation at the other site (total time in plastic bags was under 24 hours). The number of branches taken per tree was not fixed, but a minimum of 4 lichens thalli were taken from each tree to ensure the presence of enough lichen material for the duration of the study, in the event that some were removed by animals, through wind action, etc. The branches bearing the lichens were cut using pruning shears. Non-powdered latex gloves were worn at all time to prevent contamination. Each tree from which branches were removed received lichens from the other site. The lichen-

bearing branches were attached at a height of 1 - 2.5 m to the trees using clear plastic cable ties.

Twelve lichen-bearing branches were also removed at each site and reattached to branches of the 30 selected trees at the same site to construct the intrasite relocation treatment. The branches were selected according to availability of lichen material. The branches were labeled "TC".

Eight samples (each sample consisting of a complete lichen thallus) were taken randomly from each site during the initial setup to assess the background values for isotopic composition and concentration at the start of the experiment. The sulphur isotopic signals were 7.0 ± 1.0‰ and 18.3 ± 1.4‰ for BG and RH, respectively; the sulphur concentrations were 772 ± 157 ppm and 484 ± 21 ppm for BG and RH, respectively ("month 0" data, Table II.1).

Samples were taken monthly from each site starting in December 2001 and ending in May 2003 (see Table 2.2 for sampling times and period intervals). The sampling scheme was determined randomly at the start of the experiment. Using QuattroPro™, each tree/treatment combination was assigned a random number. The combinations were then sorted according to the random number assigned to them The design included 3 samples per month for each site for the local variation, and 7 in total for the intrasite and intersite relocations. The fixed number of samples for reference was deemed necessary, as no prior data on the natural background variation of the sulphur isotopic composition was available.

At collection time, each sample was labelled according to: i) time of collection (month); i) site of collection; iii) treatment; and iv) tree number. For example, 14-BG-TC7 stands for a lichen thallus collected on tree number 7 at the Botanical Garden on the fourteenth month of sampling, from a branch of the intrasite relocation treatment.

## 2.5 Sample preparation

The lichen thalli collected at each sampling location were placed in clear plastic bags and stored in a refrigerator until processed. The amount of time between sampling and the initial processing of the sample never exceeded three days. This was to minimize any storage effect and to prevent mould growth. Non-powdered latex gloves were worn at all times during the manipulation of lichen material. In the first months of sampling, colorimetric tests according to the method described by Richardson (1992) were performed on lichen thalli from both sites. This was to verify that the lichens were indeed of the *Alectoria sarmentosa* species. It was deemed necessary, especially in view of colour differences between local lichens from St. John's and Riverhead.

The first step in sample preparation was the removal of any foreign objects (conifer needles, insects, twigs, etc.) from the lichen thalli using clean stainless steel tweezers. The lichens were subsequently left to dry between two layers of clean Kimwipe™ towels to prevent contamination. Depending on the degree of hydration of the lichens at the time of collection, drying required from one to five days. There appears to be no consensus in the literature concerning the washing of the lichen samples prior to analysis. Notwithstanding

the existence of a general accord as to the necessity of removing any detritus from the lichen, it has been suggested that the washing of lichen material could result in a bias in the interpretation of the sources of sulphur stable isotopes (Wadleigh and Blake, 1999), as the sulphur removed by washing could potentially be of a different isotopic composition. An additional concern is that removing particulate material from the thallus would preferentially remove non-biological material, a distinction which was not desired in this case. Some studies have made use of washing (*e.g.*, Gonzalez *et al.*, 1998; Garty *et al.*, 2001). The samples used in this study were not washed.

The lichens were weighed once dry and brittle to the touch. The complete lichen thalli were subsequently crushed into a fine powder with liquid nitrogen (to make the tissues brittle) in an agate mortar and pestle. Before crushing, any sections which appeared to be inactive (usually brown or black and very brittle, generally becoming detached with a simple touching) were removed, along with fruiting bodies and podetia (holdfasts). The rationale for removing these was that sulphur assimilation may vary within the thallus and homogeneity is preferred (*i.e.*, cortex/medulla tissue only). The powdered lichen material was then placed into glass vials and dried once more, in an oven at 80 C for six to nine hours. Fifteen milligrams of lichen material were weighed accurately into 10 X 10 mm, 40 mg (Ultra-light) tin capsules with approximately 0.2 mg of vanadium pentoxide ($V_2O_5$) to aid combustion.

## 2.6 Sample Analysis

Sulphur isotopic composition was determined using a Finnigan MAT™-252 isotope ratio mass spectrometer at the Department of Earth Sciences, Memorial University of Newfoundland, St. John's. This instrument is interfaced with a Carlo-Erba 1500 elemental analyser. The method used for sample analysis was developed by Yun (2000). Analytical parameters for the method are given in Table 2.3.

### 2.6.1 Sulphur isotopic composition reference materials

The reference materials used for sulphur isotopic composition calibration were IAEA-S-1 ($Ag_2S$; formerly known as NZ-1) $\delta^{34}S_{VCDT} = -0.3 \pm 0.3$ ‰ and IAEA-S-2 ($Ag_2S$; formerly known as NZ-2) $\delta^{34}S_{VCDT} = +21.0 \pm 0.3$ ‰. The reference material NBS-123 (ZnS; sphalerite) $\delta^{34}S_{VCDT} = +17.09 \pm 0.31$ ‰ was used a as calibration check. The choice of isotopic reference materials was based on the expected range of $\delta^{34}S_{VCDT}$ values of +3 to +22 ‰ (Wadleigh and Blake, 1999).

### 2.6.2 Sulphur concentration reference material

The reference material used for sulphur concentration calibration was BBOT ($C_{26}H_{26}N_2O_2S$) 7.44% S w/w.

### 2.6.3 Calibration

The delta 66 values (raw measured isotopic composition) were corrected by two-point linear regression between the delta values for the two reference materials using the regression function in QuatroPro™.

Determination of total lichen sulphur content was also performed via the mass spectrometer. The area under the sulphur signal peak obtained by the spectrometer is proportionally related to the total sulphur content: by introducing different-sized samples of an elemental reference material into the system, a linear regression curve is obtained, which permits the determination of total sulphur content.

Detailed explanations of the calibration method used are presented in Appendix I.

### 2.6.4 Analytical error

The analytical error associated with the measurement of $\delta^{34}S$ was measured as 0.3‰ (n = 10). The error associated with the measurement of the sulphur content [S] was calculated as 18 ppm (n = 10).

## 2.7 Statistical analyses and regression models

All statistical analyses were performed using MINITAB™ Release 13 software.

For the purposes of this study, two criteria were considered when using *p*-values for hypothesis testing in multiple regression models. These were: i) homogeneity of the residuals (absence of any pattern); and ii) normality of the residuals. Unless otherwise

mentioned, the level of significance is $\alpha = 0.05$. Correlation coefficients are calculated as Pearson's r product-moment correlation coefficient.

Results from a similar experiment by Wiseman and Wadleigh (2002) suggested that sulphur isotopic composition and concentration observations in transplanted lichens can be explained with linear regression models. Linear regression was attempted in the course of this study, but proved to be an inaccurate way of modelling the observations. The regressions were, in most cases, not significant, and patterns could be observed in the residuals.

To detect the presence of trends in the data, the observations were plotted using LOWESS (LOcally WEighted Scatterplot Smoothing; Cleveland, 1979). LOWESS is a non-parametric exploratory smoothing technique (does not assume linearity or normality of the residuals), and as such has no simple equations or significance tests associated with it. It is an iterative procedure in which several weighted least squares equations (WLS) are fitted to the data. The squared residuals $(Y_i-\hat{Y}_i)^2$ are weighted by a factor, which is a function of $|Y_i - \hat{Y}_i|$: the distance in the Y direction between the observed $Y_i$ and the value predicted from the previous WLS regression (an iterative procedure). Observations with more variance are thus given less influence. The smoothness of LOWESS is a function of the smoothing factor f: as f is increased, more points influence the magnitude of $\hat{Y}$. The shape of the smoothing is determined solely by the data.

The use of LOWESS on sulphur concentration and isotopic composition observations revealed the presence of periodic-type trends. Multiple regression with periodic functions

was then attempted and yielded significantly better results. The regressions became significant and no patterns could be detected in the residuals.

Table 2.4 contains descriptions of the variables used in the analyses.

### 2.7.1 Multiple regression with periodic functions

The use of multiple regression methods is warranted in situations when several explanatory variables are likely to have a bearing upon the observed phenomenon (Helsel and Hirsch, 1992). The use of periodic functions in multiple regression is an efficient way of describing cyclical functions, such as seasonal trends (Helsel and Hirsch, 1992). Multiple regression with periodic functions has been used successfully in environmental studies. For example, Hirsch *et al.* (1982) used it to describe trends in water quality; variations in precipitation amounts and temperature changes affect stream flow, a situation which results in strong seasonal patterns in concentrations in surface waters (Helsel and Hirsch, 1992).

The use of periodic-type regression in this study appears justifiable, considering that sulphur concentration (and isotopic composition) - especially in urban areas- varies with seasons (Ryaboshapko, 1983). For example, Nriagu and Coker (1978) reported seasonal variation in sulphur isotope composition measurements in precipitation over the Great Lakes basin. Sulphur concentrations in inhabited areas typically increase in colder temperatures as a result of increased fossil fuel combustion. Sulphur isotopic composition, however, is a function of the source. In a region with coastal influence, seasonal variation is expected

as a result of changes in the importance of each source relative to the others or changes in inshore wind frequency.

A multiple regression with periodic functions can be described by the general equation (Helsel and Hirsch, 1992):

$$Y = \beta_0 + \beta_1 * \sin(2\pi TIME) + \beta_2 * \cos(2\pi TIME) + \beta_3 * TIME + \text{other terms} + \epsilon$$

where "other terms" are exogenous explanatory variables. Additional sine and cosine terms may be added to account for the presence of multiple cycles per year.

The amplitude A of the cycle (half the distance from peak to trough) is defined as:

$$A = (\beta_1^2 + \beta_2^2)^{1/2}$$

When binary variables are used in the regression, two functions are produced, each with an associated equation. The regression automatically leads to the two functions being in phase.

2.7.1.1 Model selection procedure

The data were fitted first at one cycle per year, and then at 2, 4, etc., cycles per year in order to determine which model would best fit the data. The significance of each new model over the simpler one was tested with nested F-tests (simpler model nested in the complex model), as follows:

$$F = \frac{(SSE_s - SSE_c) / (df_s - df_c)}{(SSE_c / df_c)}$$

where SSE is the sum of squares of the error term, df is the degrees of freedom of the error term, and the subscript letters s and c stand for simple model and complex model, respectively. If the F statistic exceeded the tabulated value of the F distribution for ($df_s$-$df_c$) at the numerator and $df_c$ at the denominator at $\alpha$=0.05, the complex model was chosen in preference to the simpler model.

Thallus weight is related to the age of the lichen and may influence uptake of nutrients and other elements. Honneger (1996) states that algal cells in the older sections of lichens are typically less active with respect to nutrient uptake compared to algal cells in the marginal sections (growing). With age, the cortex may become thicker and more compact and impede the penetration of $SO_2$ (Wirth and Türk, 1974). The use of "thallus weight" (WGT) as an additional explanatory variable was investigated for the relocation effects models using partial F-tests. With only one new explanatory variable, the partial F-test yields the same results as a t-test coefficient for the variable being tested (Helsel and Hirsch, 1992). If the computed t-statistic exceeds the critical $t_{1-(\alpha/2)}$, the new explanatory variable accounts for a significant amount of variation and should be included in the model. This was done in order to determine whether WGT added power to the equation, a situation which would warrant its presence in the regression model. The WGT variable, along with the two corresponding interaction terms WGT*TR and WGT*TIME were added to the regression model. If WGT or an interaction term improved the model significantly, the term was added to the model. Only the final regression models are presented here.

The significance of the sine and cosine terms is not discussed in the multiple regressions used in this study, as these serve only to account for seasonal variability and are calculated from the TIME variable. Triple interactions were of no concern for the analyses; For simplicity purposes, they were not included in the regression tables.

In the tables representing data from regression analyses, "SE Coef" stands for "standard error coefficient", "DF" stands for "degrees of freedom", "SS" for "sum of squares" and "MS" for "mean sum of squares".

### 2.7.2 Outliers and missing data

Suspect outliers can be identified in statistical analyses as observations falling outside of the cloud of residuals. MINITAB™ release 13 can detect outliers (observations with a large standardized residual). This feature was used for the detection of outliers in this study.

Suspect outliers were identified prior to conducting statistical analyses as $\delta^{34}S_{VCDT}$ or [S] values departing by a substantial degree from the treatment mean. The presence of suspect outliers was particularly apparent in cases where it would have been possible for lichens of two different treatments to be confused (*e.g.*, a reference thallus mistaken for a intersite or intrasite relocation thallus, due to the fact that the transplanted branches were attached to branches bearing undisturbed lichens); hence $\delta^{34}S$ or [S] values very different from the treatment mean, but close to the mean of another treatment.

Outliers were removed only when a model had been accepted. In the case of models in which WGT is used as an explanatory variable, observations with a large influence (because of very large thallus weights) were also removed.

Data were missing for one sample (13-BG-T26), due to its loss subsequent to collection.

## 2.8 Meteorological information

Weather conditions at a specific location and in a general area can affect the amount of sulphur taken up by lichens. For instance, wind direction partially determines where the sulphur originates, and wet precipitation amounts can affect lichen nutrient intake and the dissolution of sulphur compounds on the thallus (see sections 1.3.3.3 and 1.4.3.1). Krouse and van Everdingen (1984) found that $\delta^{34}S$ values varied by several per mil in soil and plants, depending upon the dominant wind direction and location with respect to sulphur sources. In view of this, it was deemed necessary to compile weather information for the general study area for the duration of the experiment.

For MUN Botanical Garden site, weather information from the St. John's airport (Environment Canada station ID: 8403503; lat. 47° 37' long. 52° 44') was used. Wind speed and direction, total precipitation and average daily temperature data were compiled for this station from November 2001 to May 2003. Wind rose diagrams for the St. John's airport were produced for each sampling period described in Table 2.2. These are included in Appendix III.

There is no weather station in Riverhead, therefore data from the Salmonier Nature Reserve weather station (station ID: 8403622; lat. 47° 16' long. 53° 17') was used for this study site. The Salmonier Nature Reserve station is located approximately 36 km north-northeast of the Riverhead study site (see Figure 1.8). Only total precipitation and average daily temperature data from this station were compiled. Wind direction and speed observations were not available.

Climatic data for both sites were compiled for each month of study, starting the day after one sampling and ending the day before the next (Table 2.2).

Table 2.1: Tree comparison data

| Tree[a] | Replicate | Weight (mg) | $\delta_{34}S$ (‰) | [S] (ppm) |
|---|---|---|---|---|
| 1 | 1 | 781.6 | 19.6 | 616 |
| | 2 | 596.0 | 17.2 | 494 |
| | 3 | 159.1 | 17.0 | 524 |
| | 4 | 188.9 | 18.7 | 624 |
| | 5 | 336.3 | 18.4 | 553 |
| | 6 | 760.0 | 18.7 | 618 |
| | Mean | **470.0** | **18.3** | **572** |
| 2 | 1 | 252.4 | 14.8 | 490 |
| | 2 | 639.7 | 16.3 | 517 |
| | 3 | 782.5 | 17.7 | 522 |
| | 4 | 225.0 | 15.6 | 566 |
| | 5 | 175.0 | 15.5 | 473 |
| | 6 | 405.2 | 17.4 | 531 |
| | Mean | **473.0** | **16.2** | **517** |
| 3 | 1 | 154.3 | 16.1 | 473 |
| | 2 | 129.3 | 16.8 | 559 |
| | 3 | 465.2 | 18.6 | 590 |
| | 4 | 363.5 | 15.2 | 527 |
| | 5 | 518.0 | 15.0 | 499 |
| | 6 | 214.1 | 18.7 | 547 |
| | Mean | **307.0** | **16.7** | **531** |
| 4 | 1 | 384.5 | 17.7 | 560 |
| | 2 | 491.9 | 17.9 | 551 |
| | 3 | 307.8 | 16.5 | 562 |
| | 4 | 400.7 | 17.4 | 573 |
| | 5 | 73.1 | 16.5 | 535 |
| | 6 | 314.6 | 16.2 | 573 |
| | Mean | **329.0** | **17.0** | **559** |
| 5 | 1 | 235.4 | 13.4 | 537 |
| | 2 | 267.2 | 16.4 | 548 |
| | 3 | 114.4 | 15.7 | 547 |
| | 4 | 569.3 | 15.6 | 549 |
| | 5 | 359.9 | 17.9 | 605 |
| | 6 | 341.8 | 17.6 | 533 |
| | Mean | **315.0** | **16.1** | **553** |

a: The trees sampled were *Picea* spp. (alive) and are located at the Riverhead site.

Table 2.2: Sampling times and corresponding experiment days for both study sites.

| Month | Site | | | | Time period |
|---|---|---|---|---|---|
| | Botanical Garden | | Riverhead | | |
| Initial sampling | 10.11.2001 | 1 | 12.11.2001 | 3 | N/A |
| 1 | 08.12.2001 | 29 | 09.12.2001 | 30 | 13.11.2001 - 07.12.2001 |
| 2 | 13.01.2001 | 65 | 13.01.2001 | 65 | 10.12.2001 - 12.01.2001 |
| 3 | 09.02.2002 | 92 | 09.02.2002 | 92 | 14.01.2002 - 08.02.2002 |
| 4 | 09.03.2002 | 120 | 09.03.2002 | 120 | 10.02.2002 - 08.03.2002 |
| 5 | 06.04.2002 | 148 | 06.04.2002 | 148 | 10.03.2002 - 05.04.2002 |
| 6 | 05.05.2002 | 177 | 05.05.2002 | 177 | 07.04.2002 - 04.05.2002 |
| 7 | 08.06.2002 | 211 | 09.06.2002 | 212 | 06.05.2002 - 07.06.2002 |
| 8 | 06.07.2002 | 239 | 06.07.2002 | 239 | 10.06.2002 - 05.07.2002 |
| 9 | 10.08.2002 | 274 | 09.08.2002 | 273 | 07.07.2002 - 08.08.2002 |
| 10 | 06.09.2002 | 301 | 07.09.2002 | 302 | 11.08.2002 - 05.09.2002 |
| 11 | 11.10.2002 | 336 | 12.10.2002 | 337 | 08.09.2002 - 10.10.2002 |
| 12 | 09.11.2002 | 365 | 09.11.2002 | 365 | 13.10.2002 - 08.11.2002 |
| 13 | 08.12.2002 | 394 | 07.12.2002 | 393 | 10.11.2002 - 06.12.2002 |
| 14 | 11.01.2003 | 428 | 11.01.2003 | 428 | 09.12.2002 - 10.01.2003 |
| 15 | 09.02.2003 | 457 | 09.02.2003 | 457 | 12.01.2003 - 08.02.2003 |
| 16 | 08.03.2003 | 484 | 08.03.2003 | 484 | 10.02.2003 - 07.03.2003 |
| 17 | 05.04.2003 | 512 | 05.04.2003 | 512 | 09.03.2003 - 04.04.2003 |
| 18 | 03.05.2003 | 540 | 03.05.2003 | 540 | 06.04.2003 - 02.05.2003 |

Table 2.3 Analytical parameters for the CF-lichen method (Yun, 2000).

| Parameter | Description |
|---|---|
| Lichen weight | 15 mg |
| Sn capsule weight | 40 mg |
| Combustion reactor temperature | 1050 C |
| Combustion reactor packing | $WO_3$, pure Cu, quartz wool |
| $O_2$ supply rate | 25-27 mL/minute |
| $O_2$ loop volume | 10 mL |
| He (carrier gas) flow rate | 80 mL/min |
| $H_2O$ trap packing | 75% $Mg(ClO_4)_2$, 25% quartz chips |
| Gas chromatograph column length | 1.2 m |
| Gas chromatograph oven temperature | 75 C |
| He pressure for $CO_2$ dilution | 25 psi |

Table 2.4: Descriptions of variables used in regression analysis.

| Variable | Type | Description |
|---|---|---|
| DELTAS | Continuous | Sulphur isotopic composition $\delta^{34}S_{VCDT}$ in ‰ (per mil) |
| SCONC | Continuous | Sulphur concentration [S] in ppm |
| TR | Categorical | Applied treatment (coded 0 or 1) |
| TIME | Continuous | Time of sampling in days from the start of the experiment |
| sin(X$\pi$TIME)[a] | Continuous | Sine function of the TIME variable |
| cos(X$\pi$TIME) | Continuous | Cosine function of the TIME variable |
| WGT | Continuous | Air dry weight of the lichen thallus in milligrams |

a: $2\pi = 0.0172$ day$^{-1}$

# Chapter 3. Results

## 3.1 Control procedures

The first two objectives of this study entailed the use of control procedures. These were incorporated into the experimental design as the local variation (reference) and intrasite relocation treatments. The former was designed to gather information on the natural variability of the sulphur isotopic composition and concentration at each site, whereas the latter was used to verify that the act of transplanting did not alter the response of lichens to changes in sulphur conditions.

Although the investigation of the transplant effect was judged a critical component of the experiment, it was not expected that the mechanics of relocation would influence lichen response to changes in sulphur conditions. This is in part because the species used in this study is an epiphyte, and therefore not reliant upon its substrate for nutrient intake, and in part because great care was taken when removing and reattaching the lichen-bearing branches. Other studies have explored the possibility of a transplant effect; in the context of those specific studies, it was found that relocating a lichen thallus did not affect its response to a change in environmental conditions (see 1.4.2.2.1).

The hypothesis of a disturbance being caused by relocation was tested with regression analysis. The treatments were included in the models as a binary variable: observations from the reference treatment were coded as 0, observations from the intrasite relocation were coded as 1. The models used were as follows (see Table 2.4 for variable descriptions):

$$DELTAS = \beta_0 + \beta_1*\sin(2\pi TIME) + \beta_2*\cos(2\pi TIME) + \beta_3*TIME + \beta_4*TR + \beta_5*TR*TIME + \epsilon$$

$$SCONC = \beta_0 + \beta_1*\sin(2\pi TIME) + \beta_2*\cos(2\pi TIME) + \beta_3*TIME + \beta_4*TR + \beta_5*TR*TIME + \epsilon$$

The interaction term TR*TIME represents the rate of change through time for the two treatments; if this term is significant, the two treatments change with time at different rates. Specific isotopic compositions and concentrations were not considered for this portion of the experiment because the aim was to determine whether or not the intrasite relocation and reference treatments were significantly different and not to investigate actual time variations in the measurements.

### 3.1.1 Botanical Garden

#### 3.1.1.1 Sulphur isotopic composition

The regression analysis for the sulphur isotopic composition data from the reference and intrasite relocation treatments at the Botanical Garden is presented in Tables 3.1 (outliers included) and 3.2 (outliers removed). The accepted model was fitted at one cycle per year.

With the removal of the outliers in the regression model, the interaction term TR*TIME became non-significant ($p = 0.050$). The sulphur isotopic composition decreased significantly with time ($p = 0.015$). The $p$-value for the TR*TIME term indicated that the slopes for the two treatments were not significantly different ($p = 0.05$), and thus that the observations from the intrasite relocation and reference treatments were indistinguishable

with regard to change through time. The observations from the two treatments were combined in a new treatment, which will be referred to as the "control treatment".

3.1.1.2 Sulphur concentration

Tables 3.3 and 3.4 give the regression model of the intrasite relocation and reference data for the sulphur concentration observations at the Botanical Garden. The model was fitted at one cycle per year.

The removal of the outliers improved the predictive capacity of the model considerably ($R^2$ increased from 19.1 to 30.0%), but did not alter the significance level of any of the terms. The interaction between time and treatment was insignificant in both models, indicating that there was no significant difference between the two treatments against time. The coefficient for TIME was significant ($p < 0.001$), and indicated an increase in the sulphur concentration over time. The observations were combined in a common treatment, designated as the "control treatment".

3.1.2 Riverhead

3.1.2.1 Sulphur isotopic composition

The results for the multiple regression analysis on the reference and intrasite relocation data for the isotopic composition at Riverhead site are given in Tables 3.5 (with outliers) and 3.6 (without outliers). The final model was fitted at one cycle per year. The removal of the outliers had no effect on the significance of any of the terms of interest (*i.e.*,

TIME, TR and TR*TIME). Based on the non-significance of the interaction term TR*TIME in the model with no outliers, the observations from the two treatments were combined in a new "control treatment".

3.1.2.2 Sulphur concentration

Tables 3.7 and 3.8 contain the results for the regression models of the sulphur concentration observations from the intrasite relocation and reference treatments at Riverhead. The model was fitted at two cycles per year. The removal of the outlier values did not affect any of the terms of interest (*i.e.*, TR, TIME, and TR*TIME).

The non-significance of the interaction term TR*TIME indicated that there was no significant difference between the two treatments. The observations from the intrasite relocation and reference treatments were combined in a "control treatment".

3.2 Relocation effects

The hypothesis of an intersite relocation effect was tested using regression analysis. Observations for the control treatment include those from reference and intrasite relocation treatments. The intersite relocation treatment will be referred to as the "transplant" treatment. Observations from the control treatment were coded as 0; those from the transplant treatment as 1. The models used were as follows:

$$DELTAS = \beta_0 + \beta_1*TIME + \beta_2*TR + \beta_3*TR*TIME + \beta_4*WGT + \beta_5*\sin(2\pi TIME) + \beta_6*\cos(2\pi TIME) + \beta_7*\sin(4\pi TIME) + \beta_8*\cos(4\pi TIME) + \beta_9*\sin(8\pi TIME) + \beta_{10}*\cos(8\pi TIME) + \ldots + \epsilon$$

$$SCONC = \beta_0 + \beta_1*TIME + \beta_2*TR + \beta_3*TR*TIME + \beta_4*WGT + \beta_5*\sin(2\pi TIME) + \beta_6*\cos(2\pi TIME) + \beta_7*\sin(4\pi TIME) + \beta_8*\cos(4\pi TIME) + \beta_9*\sin(8\pi TIME) + \beta_{10}*\cos(8\pi TIME) + \ldots + \epsilon$$

The observations from the control and transplant treatments for the two response variables DELTAS and SCONC were fitted to a multiple regression equation with periodic functions. This was carried out for both sites. Once the best model had been decided upon, the use of WGT as an explanatory variable was investigated using nested F-tests models with and without WGT (procedure described in section 2.7.1.1). Figures 3.1-3.4 represent the regression models for each variable/site combination without outlier values. Peak days are defined as the approximate day(s) of the experiment at which the periodic function cycle reaches a maximum. Changes in isotopic composition and concentration are described from the *measured* values only and were provided solely as a general indicator of the variability in the observations (measured monthly averages and modelled values did not necessarily correspond). Measured monthly means and standard deviations for isotopic composition and concentration at both sites are provided in Tables 3.17 and 3.18.

### 3.2.1 Botanical Garden

#### 3.2.1.1 Sulphur isotopic composition

Tables 3.9 and 3.10 present the regression models for sulphur isotopic composition observations at Botanical Garden, fitted at one cycle per year. Figure 3.1 shows the raw and fitted sulphur isotopic composition observations against time. Note that for clarity purposes, the data were refitted before plotting to remove the effect of weight. The refitted curve was very similar to that of the model described in Table 3.10.

The removal of the outlier values did not alter the results, but improved the prediction power of the model, as shown by the increase of the $R^2$ value from 82.7 to 89.0%. The TR*TIME term was significant, indicating that the rate of change over time differed between the treatments. The weight variable WGT was also significant and indicated that larger thalli tended to have greater isotopic compositions.

The amplitude of the cycle was 0.5 ‰ and the approximate peak day was 11 October 2002 (day 336). The measured sulphur isotopic composition for the transplanted lichens decreased from 13.5 ± 1.2 to 9.0 ± 1.4 ‰ over the study period, but did not reach the level of the control lichens, which remained fairly stable over the course of the experiment (minimum mean 5.1 ± 0.9 ‰; maximum mean 7.3 ± 1.3 ‰). The estimated time of convergence for the two curves was 1165 days, or after an additional 21 months.

3.2.1.2 Sulphur concentration

The one-cycle-per-year regression models for sulphur concentration data from the Botanical Garden are shown in Tables 3.11 (with outliers) and 3.12 (without outliers). The raw and fitted observations are plotted in Figure 3.2.

The removal of the outliers increased the $R^2$ value from 43.1% to 50.8%. TIME and TR were the only significant terms, indicating a difference between the two treatments and an increase in the sulphur concentration in both treatments over time, but no significant difference between the slopes of the two curves.

The cycle amplitude was approximately 44 ppm and peak days occurred on 9 March 2002 and 2003 (days 120 and 485). The measured sulphur concentration for the control lichens increased from 708 ± 122 to 844 ± 183 ppm, while this increase was from 638 ± 46 to 674 ± 86 ppm for the transplanted lichens. Based on the regression model, the two curves would never have converged.

3.2.2 Riverhead

3.2.2.1 Sulphur isotopic composition

Table 3.13 shows the regression model for the sulphur isotopic composition data from Riverhead with outlier values included, whereas Table 3.14 shows results for the same analysis performed on the data with the outlier values excluded. The regression model was fitted at four cycles per year. The raw and fitted observations are shown in Figure 3.3.

Only the TR and TR*TIME terms were significant. This indicated that the control and transplant treatments differed in response. Note that the removal of outlier values considerably increased the predictive power of the model ($R^2$ value increased from 75.5 to 90.3%).

Control observation means varied between 15.7 ± 1.2 ‰ and 17.8 ± 1.2 ‰ over the course of the experiment. Measured values in transplanted lichens increased from 8.4 ± 1.5 ‰ to 12.9 ± 1.4 ‰. The cycle amplitudes were 0.5 ‰ for the primary cycle, 0.4 ‰ for the secondary cycle, and 0.3 ‰ for the tertiary cycle. The largest peak occurred on 9 November 2002 (day 365). Several secondary peaks could also be observed, corresponding to secondary and tertiary cycles. The estimated time of convergence for the two treatment curves was 988 days, or after approximately 15 additional months.

3.2.2.2 Sulphur concentration

Tables 3.15 and 3.16 contain the results for the regression analysis performed on sulphur concentration observations from the Riverhead site. Figure 3.4 shows the data fitted to the model presented in Table 3.16. The model was fitted at two cycles per year.

Removing the outlier values did not alter the significance of any of the terms of interest, but increased the $R^2$ value from 39.5 to 47.3%. The TR*TIME term was significant, indicating that the rate of change differed between the treatments. Transplant observation means decreased from 615 ± 67 ppm to 517 ± 110 ppm, while control means oscillated between 438 ± 28 ppm and 700 ± 55 ppm.

The calculated cycle amplitudes were 22 and 62 ppm for the primary and secondary cycles, respectively. Peak days occurred on 9 February 2002 (day 92), 7 September 2002 (day 302), and 9 February 2003 (day 457). The estimated time of convergence for the two treatment curves was 647 days, or after approximately 3 additional months

## 3.3 Meteorological information

Figure 3.5 shows daily precipitation amounts and the 10-day running mean of average daily temperatures. On the one hand, the temperature trend over the study period was very similar for both sites, with the lowest temperatures occurring between days 60-100, and 460-500 approximately, and the highest temperatures between days 225-300. On the other hand, the precipitation patterns differed noticeably. While there was a distinct cyclical trend at the Botanical Garden, with peaks of high precipitation (partly snow) coinciding with low temperatures, and dry periods with temperature highs, no such trend could be discerned for the Salmonier Nature Reserve. Precipitation amounts at this location were fairly homogeneous over the study period. The range of temperature variation for both sites was approximately 28 C. The largest precipitation event occurred on 20 July 2002 (approximately 80 mm).

Figures III.1 - III.18 show the wind rose diagrams for each study interval. It is evident that the dominant wind provenances were west and south west. In the fourth and fifteenth intervals, however, south was also a significant direction of provenance.

Table 3.1: Model for reference and intrasite relocation treatments for sulphur isotopic composition observations at the Botanical Garden (outlier values included).

| Predictor | Coefficient | SE Coef | t | *p* |
|---|---|---|---|---|
| Constant | 6.7311 | 0.2386 | 28.21 | 0.000 |
| sin(2πTIME) | -0.0262 | 0.1642 | -0.16 | 0.873 |
| cos(2πTIME) | 0.213 | 0.1489 | 1.43 | 0.156 |
| TIME | -0.0019349 | 0.0008 | -2.49 | 0.015 |
| TR | -0.641 | 0.4085 | -1.57 | 0.120 |
| TR*TIME | 0.003362 | 0.00132 | 2.54 | 0.013 |
| | | | $R^2$ | 12.6 % |

| Source | DF | SS | MS | F | *p* |
|---|---|---|---|---|---|
| Regression | 5 | 14.125 | 2.825 | 2.65 | 0.028 |
| Error | 92 | 98.222 | 1.068 | | |
| Total | 97 | 112.346 | | | |

*Equation:* DELTAS = 6.73 - 0.026*sin(2πTIME) + 0.213*cos(2πTIME) - 0.00193*TIME - 0.641*TR + 0.00336*TR*TIME

Table 3.2: Model for reference and intrasite relocation treatments for sulphur isotopic composition observations at the Botanical Garden (outlier values removed[a]).

| Predictor | Coefficient | SE Coef | t | *p* |
|---|---|---|---|---|
| Constant | 6.7012 | 0.1884 | 35.57 | 0.000 |
| sin(2πTIME) | -0.0872 | 0.1339 | -0.65 | 0.516 |
| cos(2πTIME) | 0.2425 | 0.1182 | 2.05 | 0.043 |
| TIME | -0.0018178 | 0.0006 | -2.96 | 0.004 |
| TR | -0.4403 | 0.3226 | -0.36 | 0.176 |
| TR*TIME | 0.002107 | 0.00106 | 1.99 | 0.050 |
| | | | $R^2$ | 17.1 % |

| Source | DF | SS | MS | F | *p* |
|---|---|---|---|---|---|
| Regression | 5 | 12.1341 | 2.4268 | 3.67 | 0.005 |
| Error | 89 | 58.8069 | 0.6608 | | |
| Total | 94 | 70.9411 | | | |

*Equation:* DELTAS = 6.70 - 0.087*sin(2πTIME) + 0.242*cos(2πTIME) - 0.00182*TIME - 0.440*TR + 0.00211*TR*TIME

a: outlier values removed: 9-BG-C1, 11-BG-C2 and 15-BG-TC26

Table 3.3: Model for reference and intrasite relocation treatments for sulphur concentration observations at the Botanical Garden (outlier values included).

| Predictor | Coefficient | SE Coef | t | *p* |
|---|---|---|---|---|
| Constant | 658.57 | 32.35 | 20.36 | 0.000 |
| sin(2πTIME) | 45.2 | 22.25 | 2.03 | 0.045 |
| cos(2πTIME) | 5.44 | 20.18 | 0.27 | 0.788 |
| TIME | 0.4138 | 0.1053 | 3.93 | 0.000 |
| TR | 73.11 | 55.37 | 1.32 | 0.190 |
| TR*TIME | –0.2716 | 0.1794 | -1.51 | 0.133 |
| | | | $R^2$ | 19.1 % |

| Source | DF | SS | MS | F | *p* |
|---|---|---|---|---|---|
| Regression | 5 | 425026 | 85005 | 4.33 | 0.001 |
| Error | 92 | 1804937 | 19619 | | |
| Total | 97 | 2229962 | | | |

*Equation:* SCONC = 659 + 45.2*sin(2πTIME) + 5.4*cos(2πTIME) + 0.414*TIME + 73.1*TR - 0.272*TR*TIME

Table 3.4: Model for reference and intrasite relocation treatments for sulphur concentration observations at the Botanical Garden (outlier values removed[a]).

| Predictor | Coefficient | SE Coef | t | *p* |
|---|---|---|---|---|
| Constant | 649.45 | 28.02 | 23.18 | 0.000 |
| sin(2πTIME) | 54.31 | 19.48 | 2.79 | 0.007 |
| cos(2πTIME) | 5.89 | 17.69 | 0.33 | 0.740 |
| TIME | 0.47994 | 0.09389 | 5.11 | 0.000 |
| TR | 68.53 | 47.85 | 1.43 | 0.156 |
| TR*TIME | -0.2579 | 0.1585 | -1.63 | 0.107 |
| | | | $R^2$ | 30.0 % |

| Source | DF | SS | MS | F | *p* |
|---|---|---|---|---|---|
| Regression | 5 | 547102 | 109420 | 7.54 | 0.000 |
| Error | 88 | 1277703 | 14519 | | |
| Total | 93 | 1824805 | | | |

*Equation:* SCONC = 659 + 54.3*sin(2πTIME) + 5.9*cos(2πTIME) + 0.480*TIME + 68.5*TR − 0.258*TR*TIME

a: outlier values removed: 13-BG-C1, 14-BG-C3, 15-BG-TC26, 17-BG-C1

Table 3.5: Model for reference and intrasite relocation treatments for sulphur isotopic composition observations at Riverhead (outlier values included).

| Predictor | Coefficient | SE Coef | t | *p* |
|---|---|---|---|---|
| Constant | 16.4737 | 0.3252 | 50.65 | 0.000 |
| sin(2πTIME) | -0.363 | 0.2188 | -0.17 | 0.869 |
| cos(2πTIME) | 0.2803 | 0.2013 | 1.39 | 0.167 |
| TIME | 0.00085 | 0.00106 | 0.81 | 0.423 |
| TR | 0.5474 | 0.5809 | 0.94 | 0.348 |
| TR*TIME | -0.00147 | 0.00189 | -0.78 | 0.438 |
| | | | $R^2$ | 2.7 % |

| Source | DF | SS | MS | F | *p* |
|---|---|---|---|---|---|
| Regression | 5 | 5.017 | 1.003 | 0.51 | 0.768 |
| Error | 92 | 181.187 | 1.969 | | |
| Total | 97 | 186.205 | | | |

*Equation:* DELTAS = 16.5 − 0.036*sin(2πTIME) + 0.280*cos(2πTIME) + 0.00085*TIME + 0.547*TR − 0.00147*TR*TIME

Table 3.6: Model for reference and intrasite relocation treatments for sulphur isotopic composition observations at the Botanical Garden (outlier values removed[a]).

| Predictor | Coefficient | SE Coef | t | *p* |
|---|---|---|---|---|
| Constant | 16.7194 | 0.2535 | 65.95 | 0.000 |
| sin(2πTIME) | 0.0472 | 0.166 | 0.28 | 0.777 |
| cos(2πTIME) | 0.3695 | 0.1569 | 2.36 | 0.021 |
| TIME | 0.0001156 | 0.0008 | 0.14 | 0.887 |
| TR | 0.2914 | 0.4442 | 0.66 | 0.513 |
| TR*TIME | -0.001055 | 0.00144 | -0.73 | 0.465 |
| | | | $R^2$ | 6.9 % |

| Source | DF | SS | MS | F | *p* |
|---|---|---|---|---|---|
| Regression | 5 | 7.419 | 1.484 | 1.31 | 0.266 |
| Error | 89 | 100.541 | 1.13 | | |
| Total | 94 | 107.959 | | | |

*Equation:* DELTAS = 16.7 + 0.047*sin(2πTIME) + 0.370*cos(2πTIME) + 0.000116*TIME + 0.291*TR - 0.00105*TR*TIME

a: outlier values removed: 1-RH-C3, 6-RH-C1, 12-RH-TC19

Table 3.7: Model for reference and intrasite relocation treatments for sulphur concentration observations at Riverhead (outlier values included).

| Predictor | Coefficient | SE Coef | t | *p* |
|---|---|---|---|---|
| Constant | 471.12 | 18.52 | 25.43 | 0.000 |
| sin(2πTIME) | 16.69 | 12.42 | 1.34 | 0.182 |
| cos(2πTIME) | 32.66 | 11.68 | 2.8 | 0.006 |
| sin(4πTIME) | -21.39 | 12.93 | -1.65 | 0.102 |
| cos(4πTIME) | -64.98 | 10.96 | -5.93 | 0.000 |
| TIME | 0.19586 | 0.0599 | 3.27 | 0.002 |
| TR | -26.96 | 33.04 | -0.82 | 0.417 |
| TR*TIME | -0.0054 | 0.1069 | -0.05 | 0.960 |
| | | | $R^2$ | 43.1 % |

| Source | DF | SS | MS | F | *p* |
|---|---|---|---|---|---|
| Regression | 7 | 420772 | 60110 | 9.75 | 0.000 |
| Error | 90 | 554824 | 6165 | | |
| Total | 97 | 975596 | | | |

*Equation:* SCONC = 471 + 16.7*sin(2πTIME) + 32.7*cos(2πTIME) - 21.4*sin(4πTIME) -65.0*cos(2πTIME)+ 0.196*TIME - 27.0*TR - 0.005*TR*TIME

Table 3.8: Model for reference and intrasite relocation treatments for sulphur concentration observations at Riverhead (outlier values removed[a]).

| Predictor | Coefficient | SE Coef | t | *p* |
|---|---|---|---|---|
| Constant | 469.11 | 16.13 | 29.09 | 0.000 |
| sin(2πTIME) | 13.69 | 10.8 | 1.27 | 0.208 |
| cos(2πTIME) | 29.01 | 10.16 | 2.85 | 0.005 |
| sin(4πTIME) | -23.85 | 11.38 | -2.1 | 0.039 |
| cos(4πTIME) | -63.645 | 9.556 | -6.66 | 0.000 |
| TIME | 0.17115 | 0.05282 | 3.24 | 0.002 |
| TR | -10.15 | 28.93 | -0.35 | 0.727 |
| TR*TIME | -0.01511 | 0.09373 | -0.16 | 0.872 |
| | | | $R^2$ | 47.4 % |

| Source | DF | SS | MS | F | *p* |
|---|---|---|---|---|---|
| Regression | 7 | 341142 | 48735 | 10.83 | 0.000 |
| Error | 84 | 378014 | 4500 | | |
| Total | 91 | 719156 | | | |

*Equation:* SCONC = 469 + 13.7*sin(2πTIME) + 29.0*cos(2πTIME) - 23.8*sin(4πTIME) -63.6*cos(2πTIME)+ 0.171*TIME - 10.2*TR - 0.0151*TR*TIME

a: outlier values removed: 1-RH-C3, 4-RH-TC4, 12-RH-TC3, 13-RH-TC1, 15-RH-C1, 16-RH,C2

Table 3.9: Model for transplant and control treatments for sulphur isotopic composition observations at the Botanical Garden (outlier values included).

| Predictor | Coefficient | SE Coef | t | *p* |
|---|---|---|---|---|
| Constant | 6.1174 | 0.2559 | 23.91 | 0.000 |
| sin(2πTIME) | -0.1998 | 0.1349 | -1.48 | 0.140 |
| cos(2πTIME) | 0.4472 | 0.1276 | 3.51 | 0.001 |
| TIME | -0.0006376 | 0.0007 | -0.86 | 0.390 |
| TR | 6.6709 | 0.3591 | 18.57 | 0.000 |
| TR*TIME | -0.005641 | 0.00111 | -5.06 | 0.000 |
| WGT | 0.0009732 | 0.0003 | 3.44 | 0.001 |
| | | | $R^2$ | 82.7 % |

| Source | DF | SS | MS | F | *p* |
|---|---|---|---|---|---|
| Regression | 6 | 1297.55 | 216.26 | 144.48 | 0.000 |
| Error | 181 | 270.92 | 1.5 | | |
| Total | 187 | 1568.47 | | | |

*Equation:* DELTAS = 6.12 - 0.200*sin(2πTIME) + 0.447*cos(2πTIME) - 0.000638*TIME + 6.67*TR - 0.00564*TR*TIME + 0.000973*WGT

Table 3.10: Model for transplant and control treatments for sulphur isotopic composition observations at the Botanical Garden (outlier values removed[a]).

| Predictor | Coefficient | SE Coef | t | *p* |
|---|---|---|---|---|
| Constant | 6.2506 | 0.2203 | 28.37 | 0.000 |
| sin(2πTIME) | -0.2462 | 0.1101 | -2.24 | 0.0027 |
| cos(2πTIME) | 0.3878 | 0.1002 | 3.87 | 0.000 |
| TIME | -0.0009868 | 0.0006 | -1.72 | 0.088 |
| TR | 6.8277 | 0.2914 | 23.43 | 0.000 |
| TR*TIME | -0.0058552 | 0.0009 | -6.59 | 0.000 |
| WGT | 0.0008382 | 0.0003 | 2.62 | 0.010 |
| | | | $R^2$ | 89.0 % |

| Source | DF | SS | MS | F | *p* |
|---|---|---|---|---|---|
| Regression | 6 | 1205.57 | 200.93 | 227.69 | 0.000 |
| Error | 168 | 148.25 | 0.88 | | |
| Total | 174 | 1353.82 | | | |

*Equation:* DELTAS = 6.25 - 0.246*sin(2πTIME) + 0.388*cos(2πTIME) - 0.000987*TIME + 6.83*TR - 0.00586*TR*TIME + 0.000838*WGT

a: outlier values removed: 2-BG-T15, 3-BG-T22, 3-BG-T23, 4-BG-T15, 5-BG-T12, 8-BG-T23, 9-BG-T16, 11-BG-T24, 11-BG-T28, 11-BG-C2, 15-BG-T2, 15-BG-T6, 15-BG-TC26

Amplitude A = 0.5‰

Table 3.11: Model for transplant and control treatments for sulphur concentration observations at the Botanical Garden (outlier values included).

| Predictor | Coefficient | SE Coef | t | *p* |
|---|---|---|---|---|
| Constant | 684.08 | 22.6 | 30.27 | 0.000 |
| sin(2πTIME) | 43.73 | 13.32 | 3.28 | 0.001 |
| cos(2πTIME) | -1 | 12.62 | -0.08 | 0.937 |
| TIME | 0.31838 | 0.07319 | 4.35 | 0.000 |
| TR | -167.31 | 35.52 | -4.71 | 0.000 |
| TR*TIME | -0.0661 | 0.1099 | -0.6 | 0.548 |
| | | | $R^2$ | 43.1 % |

| Source | DF | SS | MS | F | *p* |
|---|---|---|---|---|---|
| Regression | 5 | 2018190 | 403638 | 27.56 | 0.000 |
| Error | 182 | 2665866 | 14648 | | |
| Total | 187 | 4684056 | | | |

*Equation:* SCONC = 684 + 43.7*sin(2πTIME) - 1.0*cos(2πTIME) + 0.318*TIME - 167*TR - 0.066*TR*TIME

Table 3.12: Model for transplant and control treatments for sulphur concentration observations at the Botanical Garden (outlier values removed[a]).

| Predictor | Coefficient | SE Coef | t | *p* |
|---|---|---|---|---|
| Constant | 681.58 | 20.2 | 33.73 | 0.000 |
| sin(2πTIME) | 44.28 | 11.98 | 3.7 | 0.000 |
| cos(2πTIME) | 0.99 | 11.3 | 0.09 | 0.93 |
| TIME | 0.36269 | 0.06769 | 5.36 | 0.000 |
| TR | -164.99 | 31.52 | -5.23 | 0.000 |
| TR*TIME | -0.10984 | 0.00992 | -1.11 | 0.270 |
| | | | $R^2$ | 50.8 % |

| Source | DF | SS | MS | F | *p* |
|---|---|---|---|---|---|
| Regression | 5 | 2081119 | 416224 | 36.38 | 0.000 |
| Error | 176 | 2013697 | 11441 | | |
| Total | 181 | 4094816 | | | |

*Equation:* SCONC = 682 + 44.3*sin(2πTIME) + 1.0*cos(2πTIME) + 0.363*TIME − 165*TR − 0.110*TR*TIME

a: outlier values removed: 9-BG-C1, 13-BG-C1, 14-BG-C3, 15-BG-TC26, 17-BG-C1, 18-BG-C1

Amplitude A = 44 ppm

Table 3.13: Model for transplant and control treatments for sulphur isotopic composition observations at Riverhead (outlier values included).

| Predictor | Coefficient | SE Coef | t | *p* |
|---|---|---|---|---|
| Constant | 16.5478 | 0.3257 | 50.81 | 0.000 |
| sin(2πTIME) | 0.2544 | 0.1875 | 1.36 | 0.177 |
| cos(2πTIME) | 0.3168 | 0.1818 | 1.774 | 0.083 |
| sin(4πTIME) | -0.3227 | 0.19 | -1.7 | 0.091 |
| cos(4πTIME) | 0.3347 | 0.1721 | 1.94 | 0.053 |
| sin(8πTIME) | -0.2485 | 0.1746 | -1.42 | 0.157 |
| cos(8πTIME) | 0.3687 | 0.1781 | 2.07 | 0.040 |
| TIME | 0.000317 | 0.00105 | 0.3 | 0.762 |
| TR | -7.3723 | 0.4964 | -14.85 | 0.000 |
| TR*TIME | 0.00618717 | 0.00153 | 4.05 | 0.000 |
| | | | $R^2$ | 76.8 % |

| Source | DF | SS | MS | F | *p* |
|---|---|---|---|---|---|
| Regression | 9 | 1642.81 | 182.53 | 65.41 | 0.000 |
| Error | 178 | 496.8 | 2.79 | | |
| Total | 187 | 2139.51 | | | |

*Equation:* DELTAS = 16.5 + 0.254*sin(2πTIME) + 0.317*cos(2πTIME) − 0.323*sin(4πTIME) + 0.335*cos(4πTIME) − 0.248*sin(8πTIME) + 0.369*cos(8πTIME) + 0.00032*TIME − 7.37*TR + 0.00619*TR*TIME

Table 3.14: Model for transplant and control treatments for sulphur isotopic composition observations at Riverhead (outlier values removed[a]).

| Predictor | Coefficient | SE Coef | t | *p* |
|---|---|---|---|---|
| Constant | 16.7784 | 0.2111 | 79.46 | 0.000 |
| sin(2πTIME) | 0.1397 | 0.121 | 1.15 | 0.250 |
| cos(2πTIME) | 0.5148 | 0.1175 | 4.38 | 0.000 |
| sin(4πTIME) | -0.1184 | 0.1228 | -0.96 | 0.336 |
| cos(4πTIME) | 0.3627 | 0.1116 | 3.25 | 0.001 |
| sin(8πTIME) | -0.252 | 0.1129 | -2.23 | 0.027 |
| cos(8πTIME) | 0.1464 | 0.117 | 1.25 | 0.212 |
| TIME | -0.000087 | 0.0007 | -0.13 | 0.898 |
| TR | -8.4967 | 0.3291 | -25.82 | 0.000 |
| TR*TIME | 0.008603 | 0.001 | 8.57 | 0.000 |
| | | | $R^2$ | 90.3 % |

| Source | DF | SS | MS | F | *p* |
|---|---|---|---|---|---|
| Regression | 9 | 1852.59 | 205.84 | 179.52 | 0.000 |
| Error | 173 | 198.37 | 1.15 | | |
| Total | 182 | 2050.95 | | | |

*Equation:* DELTAS = 16.8 + 0.140*sin(2πTIME) + 0.515*cos(2πTIME) − 0.118*sin(4πTIME) + 0.363*cos(4πTIME) − 0.252*sin(8πTIME) + 0.146*cos(8πTIME) − 0.000087*TIME − 8.50*TR + 0.00860*TR*TIME

a: outlier values removed: 1-RH-C3, 3-RH-T14, 4-RH-T10, 6-RH-T10, 17-RH-T18

Amplitude A (2πTIME) = 0.5‰
(4πTIME) = 0.4‰
(8πTIME) = 0.3‰

Table 3.15: Model for transplant and control treatments for sulphur concentration observations at Riverhead (outlier values included).

| Predictor | Coefficient | SE Coef | t | *p* |
|---|---|---|---|---|
| Constant | 465.66 | 16.4 | 28.39 | 0.000 |
| sin(2πTIME) | 17.534 | 9.574 | 1.83 | 0.069 |
| cos(2πTIME) | 15.338 | 9.185 | 1.67 | 0.097 |
| sin(4πTIME) | -22.256 | 9.688 | -2.3 | 0.023 |
| cos(4πTIME) | -53.03 | 8.774 | -6.04 | 0.000 |
| TIME | 0.17522 | 0.05309 | 3.3 | 0.001 |
| TR | 155.56 | 24.93 | 6.24 | 0.000 |
| TR*TIME | -0.27805 | 0.07707 | -3.61 | 0.000 |
| | | | $R^2$ | 39.5 % |

| Source | DF | SS | MS | F | *p* |
|---|---|---|---|---|---|
| Regression | 7 | 856096 | 122299 | 16.8 | 0.000 |
| Error | 180 | 13909975 | 7278 | | |
| Total | 187 | 2166071 | | | |

*Equation:* SCONC = 466 + 17.5*sin(2πTIME) + 15.3*cos(2πTIME) - 22.3*sin(4πTIME) - 53.0*cos(4πTIME) + 0.175*TIME + 156*TR - 0.278*TR*TIME

Table 3.16: Model for transplant and control treatments for sulphur concentration observations at Riverhead (outlier values removed[a]).

| Predictor | Coefficient | SE Coef | t | *p* |
|---|---|---|---|---|
| Constant | 469.09 | 14.43 | 32.52 | 0.000 |
| sin(2πTIME) | 11.845 | 8.326 | 1.42 | 0.157 |
| cos(2πTIME) | 19.08 | 8.065 | 2.37 | 0.019 |
| sin(4πTIME) | -26.144 | 8.494 | -3.08 | 0.002 |
| cos(4πTIME) | -55.967 | 7.695 | -7.27 | 0.000 |
| TIME | 0.15097 | 0.04692 | 3.22 | 0.002 |
| TR | 137.57 | 22.09 | 6.23 | 0.000 |
| TR*TIME | -0.21321 | 0.06827 | -3.12 | 0.002 |
| | | | $R^2$ | 47.3 % |

| Source | DF | SS | MS | F | *p* |
|---|---|---|---|---|---|
| Regression | 7 | 824988 | 117855 | 21.97 | 0.000 |
| Error | 171 | 917517 | 5366 | | |
| Total | 178 | 1742505 | | | |

*Equation:* SCONC = 469 + 11.8*sin(2πTIME) + 19.1*cos(2πTIME) - 26.1*sin(4πTIME) -56.0*cos(2πTIME) + 0.151*TIME + 138*TR - 0.213*TR*TIME

a: outlier values removed: 1-RH-T19, 1-RH-C3, 1-RH-TC4, 6-RH-T27, 11-RH-T25, 13-RH-T30, 15-RH-C1, 16-RH-C2, 17-RH-T25

Amplitude A (2πTIME) = 22 ppm
(4πTIME) = 62 ppm

Table 3.17: Descriptive statistics for monthly control and transplant observations at the Botanical Gardens (outliers not included).

| | Control | | | | | | Transplant | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $\delta_{34}S$ (‰) | | | [S] (ppm) | | | $\delta_{34}S$ (‰) | | | [S] (ppm) | | |
| **Day** | **n[a]** | **mean** | **SD[b]** | **n** | **mean** | **SD** | **n** | **mean** | **SD** | **n** | **mean** | **SD** |
| 1 | *8* | 7.2 | 1.0 | *8* | 708 | 122 | *NA[c]* | NA | NA | *NA* | NA | NA |
| 29 | *5* | 5.7 | 0.8 | *5* | 708 | 80 | *5* | 13.5 | 1.2 | *5* | 638 | 46 |
| 65 | *6* | 6.0 | 0.6 | *6* | 689 | 124 | *3* | 11.9 | 1.0 | *4* | 547 | 81 |
| 92 | *7* | 6.5 | 0.9 | *7* | 801 | 157 | *1* | 11.1 | NA | *3* | 618 | 85 |
| 120 | *4* | 6.4 | 0.2 | *4* | 661 | 39 | *5* | 12.6 | 1.6 | *6* | 506 | 37 |
| 148 | *6* | 6.8 | 0.3 | *6* | 827 | 19 | *3* | 12.6 | 2.0 | *4* | 676 | 45 |
| 177 | *4* | 5.9 | 1.0 | *4* | 713 | 50 | *6* | 11.9 | 1.0 | *6* | 496 | 105 |
| 211 | *7* | 5.9 | 0.9 | *7* | 730 | 131 | *3* | 10.6 | 1.0 | *3* | 545 | 40 |
| 239 | *4* | 6.6 | 0.3 | *4* | 731 | 145 | *5* | 12.0 | 0.5 | *6* | 521 | 63 |
| 274 | *5* | 7.3 | 1.3 | *4* | 709 | 145 | *4* | 11.8 | 0.6 | *5* | 562 | 69 |
| 301 | *4* | 6.0 | 0.7 | *4* | 781 | 99 | *6* | 12.4 | 1.1 | *6* | 579 | 45 |
| 336 | *3* | 6.1 | 1.3 | *4* | 874 | 137 | *4* | 11.4 | 0.7 | *6* | 650 | 72 |
| 365 | *6* | 6.9 | 0.5 | *6* | 700 | 54 | *4* | 11.2 | 0.4 | *4* | 524 | 164 |
| 394 | *5* | 6.4 | 0.5 | *4* | 902 | 201 | *5* | 12.2 | 0.6 | *5* | 498 | 33 |
| 428 | *4* | 6.1 | 0.5 | *3* | 887 | 172 | *6* | 10.2 | 0.7 | *6* | 600 | 93 |
| 457 | *4* | 6.2 | 0.9 | *4* | 963 | 104 | *3* | 10.1 | 1.3 | *5* | 765 | 76 |
| 484 | *4* | 6.1 | 0.5 | *4* | 991 | 50 | *6* | 9.5 | 0.4 | *6* | 745 | 80 |
| 512 | *5* | 5.6 | 0.4 | *4* | 801 | 176 | *5* | 9.3 | 0.5 | *5* | 670 | 37 |
| 540 | *5* | 5.1 | 0.9 | *4* | 844 | 183 | *5* | 9.0 | 1.4 | *5* | 674 | 86 |

a: number of observations
b: standard deviation
c: not applicable

Table 3.18: Descriptive statistics for monthly control and transplant observations at Riverhead (outliers not included).

| | Control | | | | | | Transplant | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $\delta_{34}S$ (‰) | | | [S] (ppm) | | | $\delta_{34}S$ (‰) | | | [S] (ppm) | | |
| **Day** | **n[a]** | **mean** | **SD[b]** | **n** | **mean** | **SD** | **n** | **mean** | **SD** | **n** | **mean** | **SD** |
| 3 | *8* | 17.0 | 1.0 | *8* | 438 | 28 | *NA[c]* | NA | NA | *NA* | NA | NA |
| 30 | *4* | 17.4 | 0.8 | *4* | 521 | 58 | *5* | 8.4 | 1.5 | *4* | 615 | 67 |
| 65 | *5* | 17 | 0.7 | *5* | 465 | 47 | *5* | 9.2 | 0.9 | *5* | 627 | 94 |
| 92 | *6* | 16.6 | 1.1 | *6* | 573 | 86 | *3* | 9.3 | 0.8 | *4* | 630 | 94 |
| 120 | *5* | 16.8 | 1.2 | *4* | 480 | 87 | *4* | 9.2 | 0.7 | *5* | 597 | 40 |
| 148 | *4* | 15.9 | 1.4 | *4* | 493 | 30 | *6* | 9.2 | 1.7 | *6* | 639 | 85 |
| 177 | *6* | 17.8 | 1.2 | *6* | 390 | 20 | *3* | 10.3 | 0.6 | *3* | 555 | 9 |
| 212 | *6* | 16.5 | 1.1 | *6* | 446 | 24 | *4* | 9.0 | 0.4 | *4* | 559 | 75 |
| 239 | *4* | 15.7 | 1.2 | *4* | 483 | 32 | *6* | 9.6 | 0.3 | *6* | 558 | 60 |
| 273 | *6* | 16.4 | 0.4 | *6* | 521 | 40 | *4* | 10.0 | 0.9 | *4* | 622 | 64 |
| 302 | *5* | 16.4 | 1.0 | *5* | 610 | 49 | *5* | 10.5 | 0.5 | *5* | 648 | 74 |
| 337 | *4* | 18.4 | 0.7 | *4* | 495 | 74 | *6* | 12.6 | 0.9 | *5* | 571 | 69 |
| 365 | *6* | 17.5 | 1.3 | *6* | 526 | 116 | *4* | 12.7 | 0.7 | *4* | 505 | 39 |
| 393 | *6* | 17.0 | 1.6 | *6* | 442 | 76 | *4* | 12.0 | 1.0 | *3* | 541 | 138 |
| 428 | *4* | 16.9 | 0.8 | *4* | 576 | 70 | *6* | 12.9 | 0.7 | *6* | 595 | 60 |
| 457 | *4* | 16.4 | 0.7 | *3* | 643 | 18 | *6* | 11.7 | 1.1 | *6* | 651 | 50 |
| 484 | *5* | 16.7 | 0.9 | *4* | 700 | 55 | *5* | 11.7 | 1.1 | *5* | 704 | 45 |
| 512 | *5* | 15.9 | 1.2 | *5* | 494 | 57 | *4* | 12.2 | 1.0 | *4* | 520 | 103 |
| 540 | *4* | 17.0 | 0.5 | *4* | 468 | 37 | *6* | 12.9 | 1.8 | *6* | 517 | 110 |

a: number of observations
b: standard deviation
c: not applicable

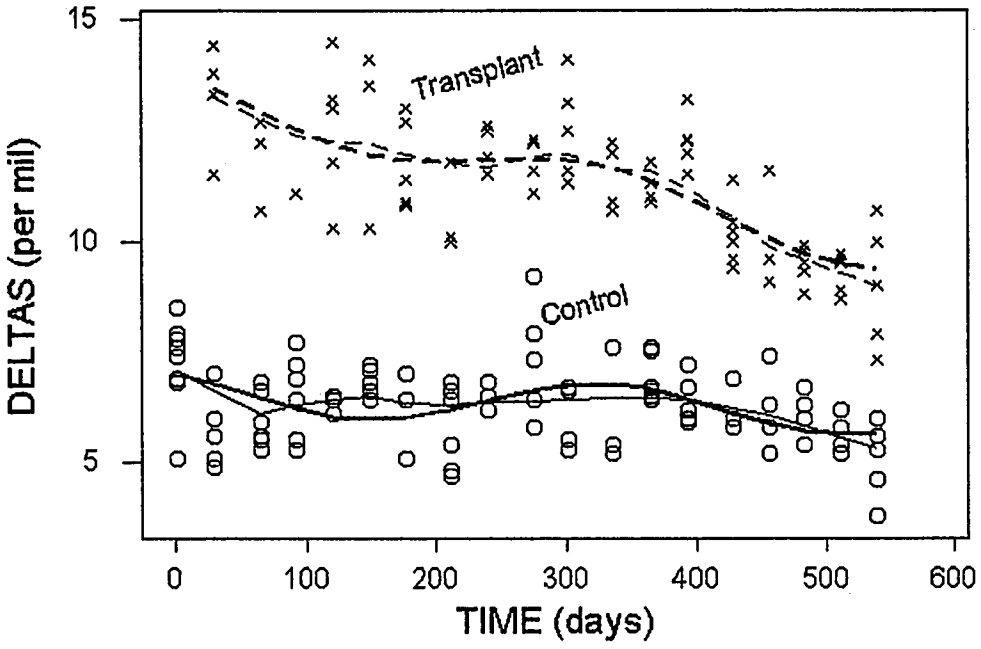



Figure 3.1: Raw and fitted observations for sulphur isotopic composition at the Botanical Gardens. Solid lines are for control observations; dashed lines are for transplant observations. Periodic function fits are represented by the bold lines: LOWESS fits are represented by finer lines (smoothing: f=0.3). Data were refitted without WGT effect for clarity purposes (results are very similar).

*Equation:* DELTAS = 6.25 - 0.246*sin(2πTIME) + 0.388*cos(2πTIME) - 0.000987*TIME + 6.83*TR - 0.00586*TR*TIME + 0.000838*WGT

Equation for refitted data: DELTAS = 6.60 - 0.285*sin(2πTIME) + 0.395*cos(2πTIME) - 0.000964*TIME + 6.84*TR - 0.00582*TR*TIME

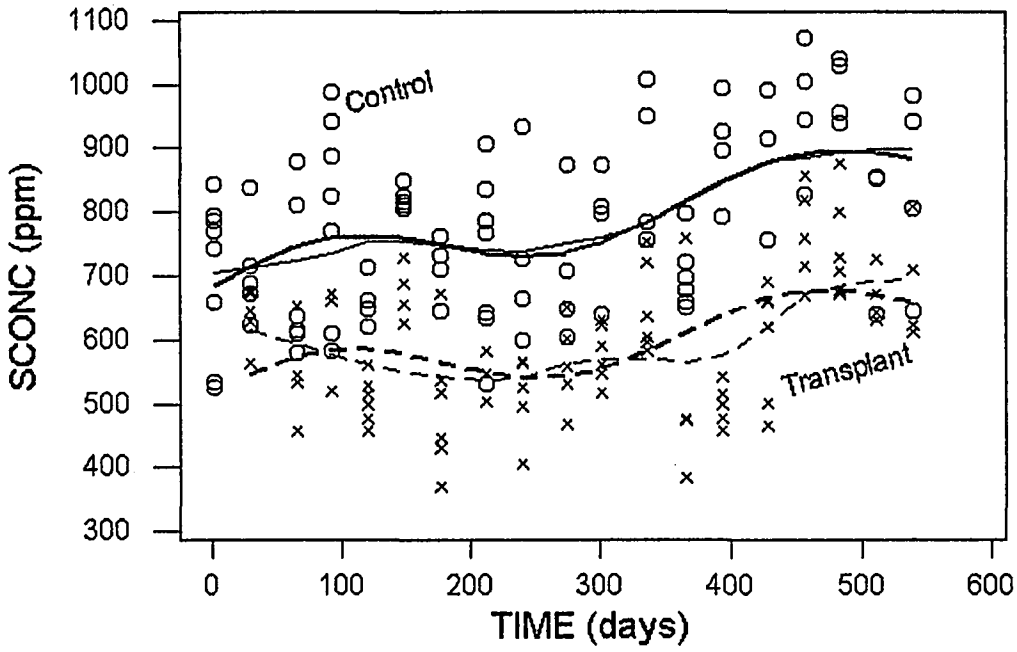



Figure 3.2: Raw and fitted observations for sulphur concentration at the Botanical Gardens. Solid lines are for control observations; dashed lines are for transplant observations. Periodic function fits are represented by the bold lines: LOWESS fits are represented by finer lines (smoothing: f=0.4).

*Equation:* SCONC = 682 + 44.3*sin(2πTIME) + 1.0*cos(2πTIME) + 0.363*TIME - 165*TR -0.110*TR*TIME

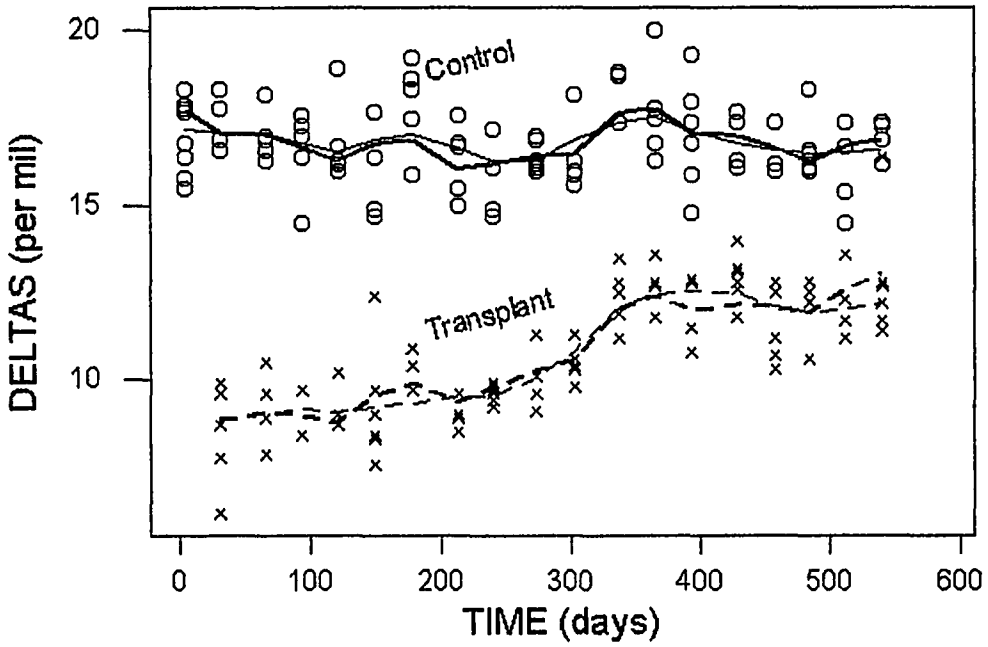



Figure 3.3: Raw and fitted observations for the sulphur isotopic composition at Riverhead. Solid lines are for control observations; dashed lines are for transplant observations. Periodic function fits are represented by the bold lines: LOWESS fits are represented by finer lines (smoothing: f=0.25).

*Equation:* DELTAS = 16.8 + 0.140*sin(2πTIME) + 0.515*cos(2πTIME) - 0.118*sin(4πTIME) + 0.363*cos(4πTIME) - 0.252*sin(8πTIME) + 0.146*cos(8πTIME) - 0.000087*TIME - 8.50*TR + 0.00860*TR*TIME

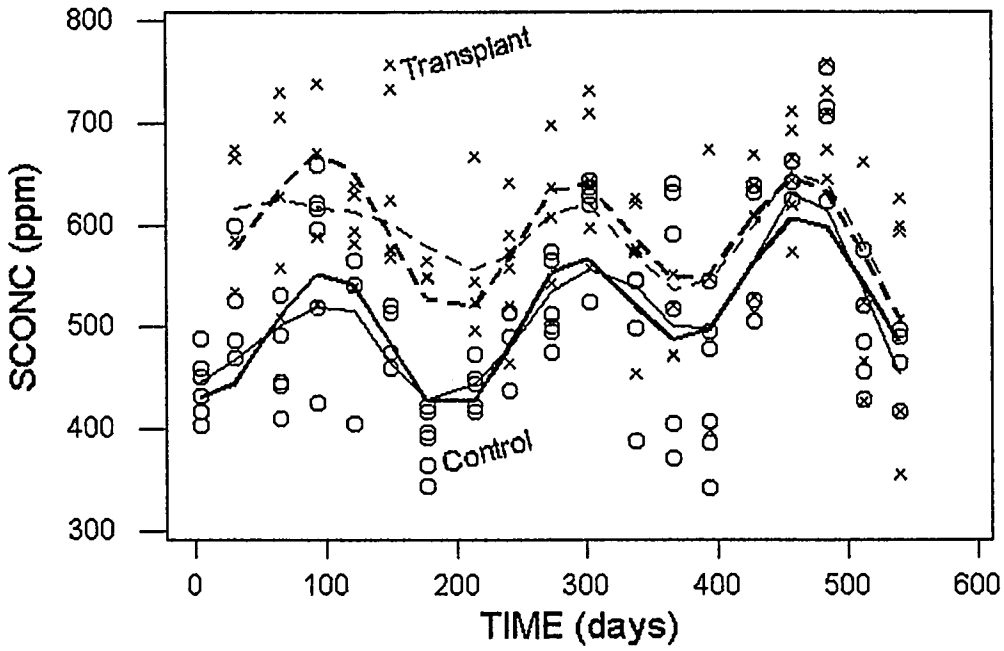
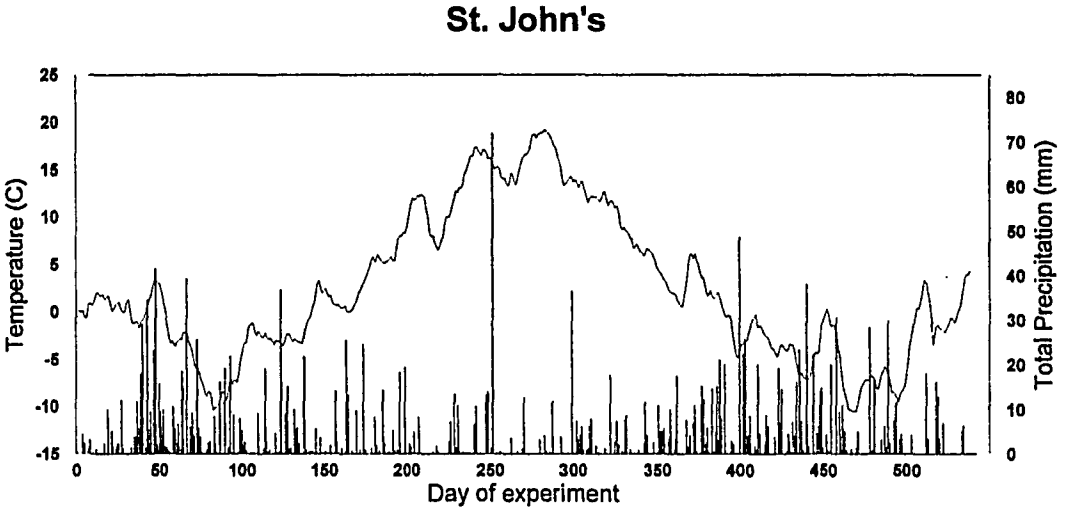



Figure 3.4: Raw and fitted observations for the sulphur concentration at Riverhead. Solid lines are for control observations; dashed lines are for transplant observations. Periodic function fits are represented by the bold lines: LOWESS fits are represented by finer lines (smoothing: f=0.2).

*Equation:* SCONC = 469 + 11.8*sin(2πTIME) + 19.1*cos(2πTIME) - 26.1*sin(4πTIME) -56.0*cos(2πTIME) + 0.151*TIME + 138*TR - 0.213*TR*TIME

a)




b)

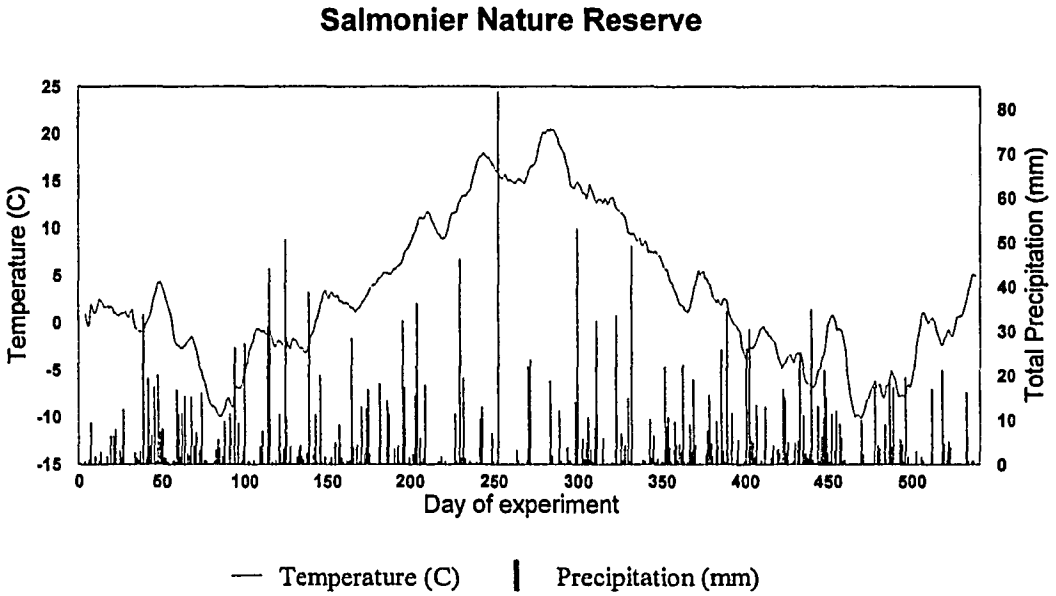



Figure 3.5: Mean daily temperatures (10-day running means, in degrees C) and total daily precipitation (mm). a) St. John's; b) Salmonier Nature Reserve.

# Chapter 4. Discussion

There is limited information on the influence of transplant on sulphur isotopic composition measurements in lichens. In fact, we are aware of only one study that addressed this question (Wiseman and Wadleigh, 2002). In addition, lichen transplant studies have traditionally been performed from unpolluted to polluted locations, hence little information is available on lichen response to *improvements* in sulphur conditions. Finally, the literature on studies dealing with rates of sulphur accumulation in lichens remains, at best, indefinite. The results discussed here have provided valuable information necessary to answer these questions.

## 4.1 Control procedures

With regard to the initial study objective, it was found that the mechanics of relocation did not affect lichen responses to changes in sulphur isotopic composition or concentration, as expected. Regression analyses performed on data from reference and intrasite relocation treatments indicated there was no significant difference between the two treatments. The results were concordant with the findings of Garty *et al.* (1993), Zambrano *et al.* (1999), Zambrano and Nash (2000) and Wiseman (1999). The epiphytic nature of *Alectoria sarmentosa* and the fact that substrates were relocated with the lichen thalli probably precluded disturbances that would have altered lichen responses to the changes in sulphur conditions.

## 4.2 Relocation effects and natural variability

The use of multiple regression with periodic functions for modelling the observations revealed cyclicity in isotopic composition and concentration measurements. A number of studies have shown that pollutant measurements in lichens provide good estimates of atmospheric pollutant concentrations (*e.g.*,, Gailey *et al.*, 1985; Zakshek *et al.*, 1986), but this is the first study, to the best of our knowledge, in which periodic-type trends in lichen pollutant concentrations were uncovered. Most biomonitoring studies have been chiefly concerned with total accumulation time of pollutants, and with the effects of these pollutants on lichen distribution patterns and physiology. The results obtained here suggested the existence of two response time scales: short-term (periodic cycles) and long-term (convergence of curves). At first glance, this may appear to conflict with existing knowledge, which suggests that relatively long periods of time are necessary for transplanted lichens to reflect local pollutant conditions. For instance, Hale and Lawrey (1985) studied lead accumulation by *Parmelia baltimorensis*; peak values were reached within a year. And Wiseman and Wadleigh (2002) showed that 18 months would have been necessary for lichens transplanted to an urban location to reach local sulphur conditions. Another potential cause for doubting the possibility of detecting cyclical trends could be the fitting of observations to a multiple regression equation with periodic functions, which forces the two treatment curves to be in phase (even though they have different equations). It could be hypothesized that the correspondence between cyclical highs and lows (short-term response) between control and transplant observations was a fictitious product of the regression.

However, results from the use of LOWESS -a non-parametric smoothing technique- in conjunction with the parametric regression models, also revealed periodic-type trends and showed that periods of high and low isotopic compositions and concentrations were indeed concomitant between the treatments (Figures 4.1 - 4.4). The LOWESS curves followed the sinusoid curves closely, adding yet more weight to the argument of cyclical trends, and to the possibility of measuring short-term responses in lichens.

### 4.2.1 Climatic effects

Because lichens are able to photosynthesize even at extreme temperatures (up to 70 C for some species: Lange, 1953), ambient temperatures were not expected to have a direct effect on sulphur absorption by lichens. This, of course, was notwithstanding weather-related considerations on wetting and drying cycles. An important consideration in urban and industrial areas, however, is the influence of weather conditions on human activities, and by extension on atmospheric sulphur conditions. Increased fossil fuel combustion in winter for home-heating purposes results in an augmentation of sulphur dioxide emissions. This influences not only the atmospheric sulphur concentration, but also the isotopic signal (by effecting a change in the relative importance of anthropogenic sources to natural ones).

The influence of climate is such that in an urban area with oceanic influence, one would expect to measure the lowest atmospheric sulphur isotopic compositions and highest concentrations during periods of cold weather, when natural sources of sulphur can be partially eclipsed by anthropogenic sources. In a coastal atmosphere, natural sources have

typically greater isotopic signatures than urban sources, due to the presence of sea spray sulphates ($\delta^{34}S$ + 21 ‰; Rees *et al.*, 1978). An increase in anthropogenic sulphur dioxide emissions would thus lead to an increase in sulphur concentration, but a decrease in the isotopic composition.

The geographical situation of the Riverhead site would, at any rate, presumably minimize the influence of sulphur emissions from urban locations and the refinery. Likewise, Wadleigh and Blake (1999) found no evidence of significant long-range transported sulphur in isotopic composition and concentration measurements in *A. sarmentosa* thalli collected throughout the island of Newfoundland.

### 4.2.2 Sulphur isotopic composition

Results from the Botanical Garden site supported the assumption of anthropogenic influence. The lowest $\delta^{34}S$ values in the control observations occurred between days 110 and 180, and after day 490 of the experiment (Figure 4.1). This roughly corresponded to temperature lows observed between days 70-140 and 450-510. The greatest $\delta^{34}S$ values in the control observations occurred between days 300 and 350, in some agreement with temperature highs observed between days 220-310. If a lag of 40-70 days between temperature variations and the response in isotopic composition was admitted, the agreement between temperature patterns and isotopic composition measurements improved significantly. Low isotopic compositions were observed during winter months, when anthropogenic sulphur emissions were more important.

The case of the Riverhead site was not as straightforward. A characteristic feature of the isotopic composition model was the presence of two highs in isotopic composition, but the absence of any distinct lows. If the sulphur isotopic composition cycles were assumed to have been associated with nearby or distant anthropogenic emissions, high $\delta^{34}S$ values would have tended to be associated with warmer temperatures, and lows with cold periods. There appeared to exist some agreement between minor lows in the regression model (days 120 and 484) and periods of cold weather (days 70-110 and 460-500; Figure 4.3). The lag period between the start of colder temperatures and its detection in the isotopic signal was between 30 and 50 days approximately, similar to the lag observed at the Botanical Garden. Some possible sources for pulses of low isotopic composition sulphur may have been the widespread use of all terrain vehicles in the site area and anthropogenic sulphur from surrounding towns. As well, despite the dominant westerly winds, it is possible that some anthropogenic emissions were carried to Riverhead from the north and northwest. This is supported by the presence in the wind rose diagrams of peaks from those directions (Figures III.1 - III.18).

The short-term response times observed in isotopic composition at the two sites were probably related to rates of sulphur exchange within lichen thalli. Sulphur isotope measurements certainly reflected both rates of incoming (from the atmosphere) and outgoing (lost during precipitation events) sulphur. Maynard *et al.* (1984) determined that although the majority of total sulphur in plants is usually in the organic forms of amino acids and sulpholipids, inorganic sulphate may become the major sulphur form when excess sulphur

is available. Krouse (1977), Takala *et al.* (1985), and Häffner *et al.* (2001) showed that change in sulphur concentration in lichens transplanted to heavily polluted sites was mainly attributable to changes in the inorganic sulphur fraction (sulphate). Wiseman and Wadleigh (2002) demonstrated that sulphur isotopic composition measurements in lichens transplanted to an urban location could not be described by simple mixing. The distinction between inorganic and organic S was not made here, but it is probable that it would have provided useful information on the cyclicity observed in the measurements. Total cyclical variations in isotope composition measurements in control lichens were small (1‰) and probably smaller than actual seasonal variation in atmospheric sulphur.

The lag between temperature and isotopic composition measurements could have been a function of the time necessary for sulphur to be exchanged in the lichen tissues. Wiseman and Wadleigh (2002) calculated that after a 12-month exposure time, the proportion of sulphur in the transplanted lichens originating from the atmosphere at the transplant site was 64%, indicating that at least some of the sulphur originally present in the transplanted lichens had been replaced. Lichens are known to store the sulphur-containing products of photo- and mycobionts, such as free amino acids and proteins (polyols, and polysaccharides) (Gorin *et al.*, 1988); biologically-bound sulphur would probably remain in the thallus longer compared to inorganic sulphur. Memory effects from episodes of high sulphur concentration are also a possibility. Reis *et al.* (2002) calculated that the "remembrance time" in lichens for high sulphur availability conditions was 64 years when

calibrated to average conditions, and 0.38 years when calibrated to maximum concentrations (pulses of high sulphur concentration).

An important difference between the two sites was the presence in the Riverhead model of three significant pairs of sine/cosine terms, which indicated that secondary cycles were detectable (Table 3.14). This suggested influence from mostly non-anthropogenic sources of sulphur, as it is unlikely that such complex human-related cycles of sulphur emissions were measurable in the relatively unpolluted area of Riverhead but not in the urban area of St. John's. However, without additional information, it is difficult to determine the nature of these sources. A possibility is that sulphur of biogenic origin contributed to lows in isotopic composition. The Riverhead sampling location was located approximately 500 m from the entrance to St. Mary's Bay. Sulphur of low isotopic composition produced during biological processes in the vicinity probably contributed to cyclical variations. This would have added to the difference between the expected $\delta^{34}S$ value of + 21 ‰ for sea spray sulphates and the average value over the course of the experiment (16.8 ± 1.4 ‰ in control lichens). Sea marshes and the tidal flats of marine environments are areas of intense biological activity that serve both as sources and sinks for a number of sulphur compounds. Sulphur plays an important role in biological processes in these environments (Ingvorsen and Jørgensen, 1982). $H_2S$ is the major sulphur product, but other volatile sulphur compounds, such as DMS, DMDS (dimethyldisulfide; $CH_3S_2CH_3$), $CH_3SH$ (methyl mercaptan), COS and $CS_2$ have been identified (Aneja *et al.*, 1979; Hill *et al.*, 1978). Biogenic sources tend to have $\delta^{34}S$ values less than 0 ‰ (Krouse *et al.*, 1991). Without the presence of significant human

and industrial activity in St. John's, the two locations would probably have shown similar seasonal patterns.

Lichens transplanted to the Botanical Garden responded to the new conditions, as indicated by the gradual decrease in isotopic composition, but did not reach isotopic compositions typical of locally-growing lichens. In the case of Riverhead, isotopic composition measurements also revealed a lichen response to the change in sulphur conditions, but again, the 18-month period was not sufficient for the transplanted lichens to become indistinguishable from local ones. In contrast, results from Wiseman and Wadleigh (2002) suggested that 18 months would have been sufficient for lichens transplanted from a marine location to the MUN Botanical Garden to represent local conditions. The average difference in sulphur isotopic composition between the two sites was, however, approximately 8 ‰, compared to an average difference of 11 ‰ in this experiment. Lichens submitted to larger changes in sulphur conditions may require longer adaptation periods. Discrepancies between the two experiments may be also be due partly to the fact that Wiseman and Wadleigh (2002) used linear regression models, which by their very nature cannot account for seasonal variation. Additionally, the mathematical convergence of sinusoid curves is likely to occur after longer periods of time compared to linear functions, because of cyclicity. In view of this, it is not unexpected that 18 months were insufficient for the transplanted lichens to become indistinguishable from local ones.

Wiseman and Wadleigh (2002) also showed that the isotopic composition measured in lichens transplanted to an urban location was not a function of simple mixing. The

simultaneous increase in sulphur concentration (Figure 4.4) and decrease in isotopic composition (Figure 4.3) in lichens transplanted to the Botanical Garden indicated that both uptake (change in isotopic composition) and loss of sulphur (decrease in concentration) were taking place simultaneously. Sulphur present in the lichens at the start of the experiment was lost during precipitation events and replaced, at least in part, by sulphur from sea spray sulphates available from the surrounding atmosphere. We assume that the majority of sulphur leached from the thallus was non-biological sulphur (*i.e.*, sulphates).

Precipitation sulphate measured in St. John's had an average $\delta^{34}S$ value of + 7‰ (Jamieson, 1996), a value similar to isotopic composition measurements for modelled observations for the control treatment lichens at this site (min. 5.6 ‰ , max 7.0 ‰: Figure 4.1). Krouse (1977; 1980) and Winner *et al.* (1978) found that ASR caused $\delta^{34}S$ values measured in plants and lichens to be typically 1.5 ‰ lighter than the sulphur source. The difference between minimum isotopic composition measurements and average $\delta^{34}S$ values in rain of 1.4 ‰ could have been a result of ASR. Had the organic and inorganic sulphur fractions been distinguished, we would have been able to better quantify this effect. Biologically-bound sulphur is probably subject to more fractionation than inorganic sulphur, as the presence of the latter is mostly a result of passive processes.

The thallus weight variable WGT was present in the isotopic composition model for the Botanical Garden and the coefficient was positive (Table 3.10), indicating that larger lichens tended to have greater isotopic compositions. The correlation between isotopic composition and concentration in the control lichens was significant ($r = -0.440$, $p < 0.001$;

Table 4.1), as it was for weight and isotopic composition ($r = 0.207$; $p = 0.041$). In lichens growing at the same site, larger thalli (larger weight) would presumably be older. As algal cells in the older sections of lichens are less active with respect to nutrient uptake and photosynthesis than algal cells in the newer sections (Honneger; 1996; Maguas and Brugnoli, 1996; respectively) and the cortex in older sections can also thicken and prevent or impede the penetration of $SO_2$ (Wirth and Türk, 1974), the greater isotopic composition in the larger lichens could be related to differential absorption in new and old sections. Larger lichens may have contained more "old" sulphur. Yun (2000) found that sulphur isotopic compositions and concentrations differed within *Alectoria sarmentosa* thalli collected near the Come-by-Chance oil refinery with older portions typically showing greater isotopic compositions. Mean monthly sulphur concentrations at the Botanical Garden site increased during the course of the experiment from 708 ± 122 to 844 ± 183 ppm (Table 3.17). The average sulphur concentration in lichens measured by Wiseman and Wadleigh (2002) in 1997 - 1998 was 500 ± 74 ppm. These results suggest that anthropogenic emissions and the proportion of anthropogenic to natural sulphur are increasing in the urban area of St. John's, Newfoundland and Labrador; "older" sulphur would thus have a greater average isotopic composition. Indeed, weight and isotopic composition were correlated only in the control lichens at this site, although WGT was significant for the model as a whole. As expected, such an effect of increased sulphur dioxide emissions was not found at the coastal site of Riverhead; isotopic composition and weight in the control lichens were not significantly correlated (Table 4.1). In contrast, higher isotopic compositions in lichens transplanted to

the Riverhead site were associated with lower concentrations and smaller thallus weights ($r = -0.358, p = 0.001$ and $r = -0.239, p = 0.023$, respectively; Table 4.1). This suggests that the exchange of sulphur in transplanted lichens occurred faster in smaller thalli. Häffner *et al.* (2001) showed that sulphur accumulation in lichens exposed to $SO_2$ was mainly due to the accumulation of inorganic sulphate. If inorganic sulphate is accumulated at a faster rate, the converse would also be true: biologically-bound sulphur of anthropogenic origin would be lost more slowly than inorganic sulphur. Larger lichens would likely have had higher amounts of anthropogenic sulphur at the start of the experiment and the change in isotopic composition would have been less evident.

### 4.2.3 Sulphur concentration

As in the case of isotopic composition measurements, seasonal trends (*i.e.*, short-term responses) were detectable in lichens at both sites. At the Botanical Garden, the temperature high observed between days 225 and 325 (Figure 4.2) was in agreement with a low in sulphur concentration observed between days 210 - 290. Additionally, colder periods corresponded with periods of higher sulphur concentrations (days 80 - 150 and 425 - 530). The agreement between expected lows and highs in concentration and temperature trends was greater than for isotopic composition measurements, but the predictive power of the model was lower ($R^2$ = 50.8%). At Riverhead, the regression model indicated the presence of an anthropogenic effect similar to that observed in the urban location, as suggested by the agreement between concentration highs (between days 99-120 and 457-

484) and temperature lows (Figure 4.4). The central high (days 273-302), however, occurred in the summer period. Results suggested that some anthropogenic emissions were present in the atmosphere surrounding Riverhead, but that another source of sulphur was dominant during the summer months. Wadleigh and Blake (1999) found no evidence of long-range-transported sulphur in the atmosphere of Newfoundland; emissions from the Canadian mainland are thus improbable. Results for isotopic composition indicated that an anthropogenic effect, if present at all, did not clearly set apart winter and summer isotopic compositions. It is probable that the change in concentration was large enough to affect sulphur concentration measurements in lichens, but that the difference in isotopic signal was not easily detected. Note in Figure 4.4 that the amplitude of the variation in sulphur concentration was quite large compared to that of the other models (Figures 4.1 - 4.3) and that the maxima attained in sulphur concentration were comparable to concentrations measured in control lichens at the Botanical Garden. As for the increase in sulphur concentration in the summer period, it could have been a result of biogenic emissions of sulphur gases, as was suggested for sulphur isotopic composition measurements at Riverhead. Much land in the area around the Riverhead site is under the effect of tidal action, and sea marshes and the tidal flats of marine environments are known to be significant sources of biogenic sulphur compounds, especially in warmer temperatures, as a result of increased biological activity (Hill *et al.*, 1978; Aneja *et al.*, 1979; Ingvorsen and Jørgensen, 1982). The peak in sulphur concentration in Figure 4.4 fell precisely during periods of warmer temperatures, which would be concordant with these findings.

The correspondence between highs/lows and temperature patterns was similar for the two Botanical Garden models, allowing a lag of about 50 days in isotopic composition. The absence of a lag period in sulphur concentration is comprehensible, as the total sulphur concentration is independent of the sulphur form (organic or inorganic), and thus from rates of sulphur exchange within the thallus. Wet precipitation events may have caused leaching of particulate sulphur, which was being replenished by a continuous supply of anthropogenic sulphur. Results agree with previous studies that suggested that sulphur accumulation in lichens provides a reasonably accurate estimate of $SO_2$ concentration in the atmosphere (Gilbert, 1973; Puckett and Fineman, 1980; Hopp and Kappen, 1981; Richardson and Nieboer, 1983; Levin and Pignata, 1995).

The expected response for the transplanted lichens was a gradual change in sulphur concentration toward control values (Wiseman and Wadleigh, 2002). This was observed at the Riverhead site, but at the Botanical Garden, however, the sulphur concentration model (Figure 4.2) showed a gradual increase in calculated sulphur concentration over time in both treatments and indicated that the curves for the two treatments would never have met (distance between curves increased with time). Also, the increase in sulphur concentrations was such that the time-trends for the two treatments were not significantly different. It is probable that the increase in ambient sulphur concentrations over the course of the experiment at this site was sufficient to obscure any difference in trend between control and transplant lichens. Sulphur accumulation in lichens has been shown to be directly dependent on the $SO_2$ level of the exposure (Leblanc and Rao, 1973) and sulphur concentration

measurements in lichens between 1997 and 1998 were considerably lower (500 ppm average) (Wiseman and Wadleigh, 2002). This could also have been a factor at the Riverhead site, as sulphur concentrations in the control lichens increased over the course of the experiment. Regressions were based on 18 months of observations; it is probable that a longer time frame would have revealed additional trends.

Sulphur absorption may be a function of morphological characteristics. For example, thallus age may have been a factor in the larger variability observed in sulphur concentration measurements compared to isotopic composition, and consequently to the lower predictive powers of the sulphur concentration models. Maguas and Brugnoli (1996) showed that carbon discrimination was greatest in younger or marginal sections of thalli for seven lichen species.

In contrast to the isotopic composition model, the regression model for sulphur concentration at the Botanical Garden did not include WGT as an explanatory variable. This was concordant with our supposition that the weight effect was related to different rates of sulphur absorption in old compared to young portions of lichen thalli, as sulphur concentration measurements cannot distinguish between old and new sulphur.

### 4.2.4 Additional considerations

A striking result was the presence of significant secondary and tertiary cycles in models for the Riverhead site, but not for the Botanical Garden (Figures 3.1 to 3.4). It is logical to hypothesize that this was due, at least in part, to the existence of significant

anthropogenic emissions in the Botanical Garden area. These emissions had isotopic signatures significantly lower than natural sources and were probably eclipsing the higher $\delta^{34}S$ values of natural sources.

We hypothesized that lichens originating from the Botanical Garden would show a slower rate of response compared to those from Riverhead, possibly as a result of physiological damage; this was not borne out by the results. The regression models for isotopic compositions indicated that convergence for transplant and control treatments would have occurred on the 1165$^{th}$ day of the experiment at the Botanical Garden, and on the 988$^{th}$ day at Riverhead, a difference of approximately 6 months. The regression models for sulphur concentration at the two sites could not be compared due to significant increases in ambient sulphur concentrations at the Botanical Garden.

Qualitative observations suggested the presence of some physiological damage in the control lichens at the Botanical Garden site throughout the duration of the experiment, and in lichens transplanted to that site (especially during last 4 months of experiment). Lichens exhibited a distinct dull grey-green colour and were more brittle than lichens originally growing in Riverhead. In a majority of cases, inactive sections of the thallus were present (dry, brown brittle sections). In a study on the physiological responses of 4 lichen species transplanted along an $SO_2$ pollution gradient, Häffner *et al.* (2001) found that bleaching occurred as early as 4 weeks following the onset of exposure and that colour change was linked to chlorophyll destruction or loss (also Moser *et al.*, 1983). Qualitative information on physiological parameters would be required to assess the extent of injuries. If injury did

occur, it is possible that its effect on sulphur absorption and exchange capacity in lichens was offset by other factors, such as the difference in the ratio of incoming to outgoing sulphur, which was likely much larger at the Botanical Garden due to higher concentrations of sulphur in the surrounding atmosphere.

The observations for sulphur concentration in the control treatment were consistent with results of previous studies, which showed that lichens located closer to urban centres tend to have greater sulphur concentrations (Nieboer and Richardson, 1981; Wadleigh and Blake, 1999). The average sulphur concentration over the course of the experiment was 776 ± 152 ppm at the Botanical Garden and 511 ± 100 ppm at Riverhead (all control observations combined). The relative difference in mean sulphur concentrations between the two sites was small compared to the difference in sulphur isotopic compositions. In addition, the predictive powers of the regression models for sulphur concentration were considerably lower than those for isotopic composition. Because each model included data from two treatments, the larger disparity in isotopic composition between the two treatments probably increased the predictive powers. The inherent variability in measured values was greater for sulphur concentration measurements (17% in $\delta^{34}S$ values and 20% in [S] for control lichens at BG; 8% in $\delta^{34}S$ values and 20% in [S] in control lichens at RH).

The overall increase in background sulphur concentrations at the Botanical Garden precluded any comparison between the rates of sulphur accumulation/loss (sulphur concentration) in the transplanted lichens between the two sites.

The expected time of convergence of approximately 21 months for sulphur concentration observations at Riverhead between control and transplanted lichens was longer than the range of times determined by transplant experiments described in the literature. However, other studies focussed on investigating the rate of sulphur accumulation, as opposed to sulphur loss. In the case of sulphur isotopic compositions, the results can only be compared with those from a study by Wiseman and Wadleigh (2002) in which it was reported that transplanted lichens would have required 18 months to reach local conditions. The required times, as determined in this experiment, were much longer: 33 and 38 months for Riverhead and the Botanical Garden, respectively. This was probably partly due to the larger initial difference in isotopic composition between the two sites.

## 4.3 Implications for biomonitoring

One of the common uses of active biomonitoring is the assessment of pollutant levels at a given site. Lichens are taken from a "clean" location and relocated to one where the atmosphere is known to be contaminated by a certain pollutant. These studies typically last less than two years. The present study showed that total adaptation times may be much longer than thought before and that a portion of the observed change in pollutant levels may be due to seasonal variation, which cannot be accounted for by the use of modelling techniques such as simple linear regression. It may be necessary to account for climate-related cycles -especially in urban areas- in order to obtain an accurate representation of occurring phenomena. Likewise, using only initial measurements of pollutant concentrations

for baseline data may introduce significant error, as levels may vary a great deal between seasons; knowledge of pollutant levels in undisturbed lichens throughout the course of the experiment may prove necessary. In addition, when considering total response time, the extent of the disparity between original and new conditions is likely a factor. A longer response time is probably necessary for larger differences.

A significant finding concerns the ability of lichens to release some of the sulphur in their tissues. Many previous authors have indeed assumed that lichens can accumulate elements and retain them indefinitely. This is obviously not the case, at least with sulphur, and probably with other elements. We did not identify the nature -organic or inorganic- of the sulphur leached from the lichen thalli, but it is probable that a majority of the sulphur eliminated from the tissues was in the inorganic form. In calculating accumulation rates in lichens, amounts lost during precipitation events or by other means must also be accounted for, hence the need to consider local climatic conditions.

The use of isotopes in conjunction with concentration measurements in the case of sulphur, and perhaps of other elements, permits a more comprehensive analysis of pollutant sources and natural cycles than parameters used individually. When investigating sulphur in lichen thalli, distinguishing between organic and inorganic fractions of sulphur may provide invaluable information.

Table 4.1: Pearson r product-moment correlation coefficients and *p*-values (below, italicized) for DELTAS, SCONC and WGT in control and transplanted lichens.

| | | **Botanical Garden (BG)** | | | **Riverhead (RH)** | | |
|---|---|---|---|---|---|---|---|
| **Treatment** | **Variable** | **DELTAS** | **SCONC** | **WGT** | **DELTAS** | **SCONC** | **WGT** |
| *Control* | **DELTAS** | 1 | | | 1 | | |
| | | - | | | - | | |
| | **SCONC** | -0.440 | 1 | | -0.196 | 1 | |
| | | *<0.001* | - | | *0.053* | - | |
| | **WGT** | 0.207 | -0.155 | 1 | 0.065 | -0.236 | 1 |
| | | *0.041* | *0.128* | - | *0.525* | *0.019* | - |
| *Transplant* | **DELTAS** | 1 | | | 1 | | |
| | | - | | | - | | |
| | **SCONC** | -0.415 | 1 | | -0.358 | 1 | |
| | | *<0.001* | - | | *0.001* | - | |
| | **WGT** | 0.149 | 0.012 | 1 | -0.239 | -0.030 | 1 |
| | | *0.160* | *0.910* | - | *0.023* | *0.780* | - |

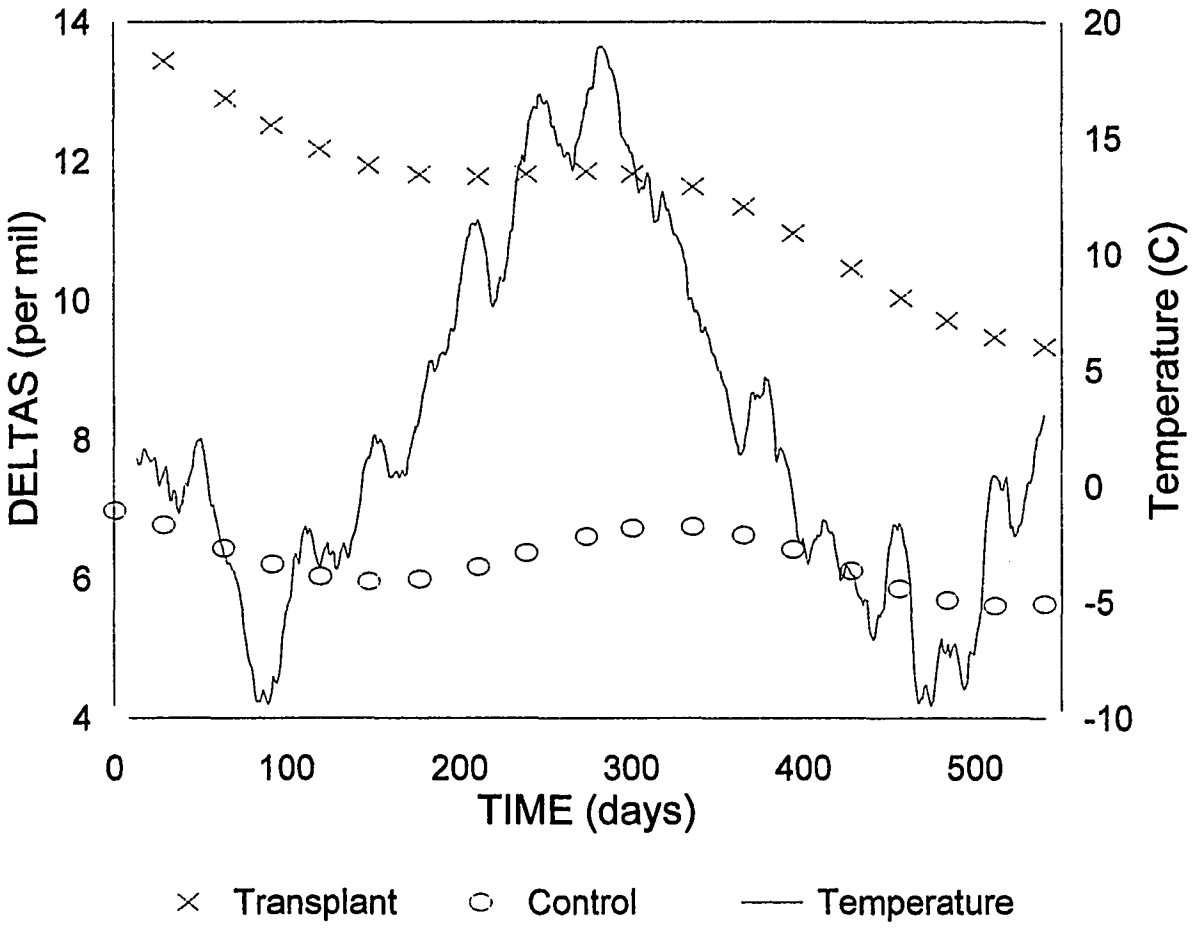



Figure 4.1: Regression model for sulphur isotopic composition at the Botanical Gardens (Table 3.10) and mean daily temperature (degrees C; 15-day running mean).

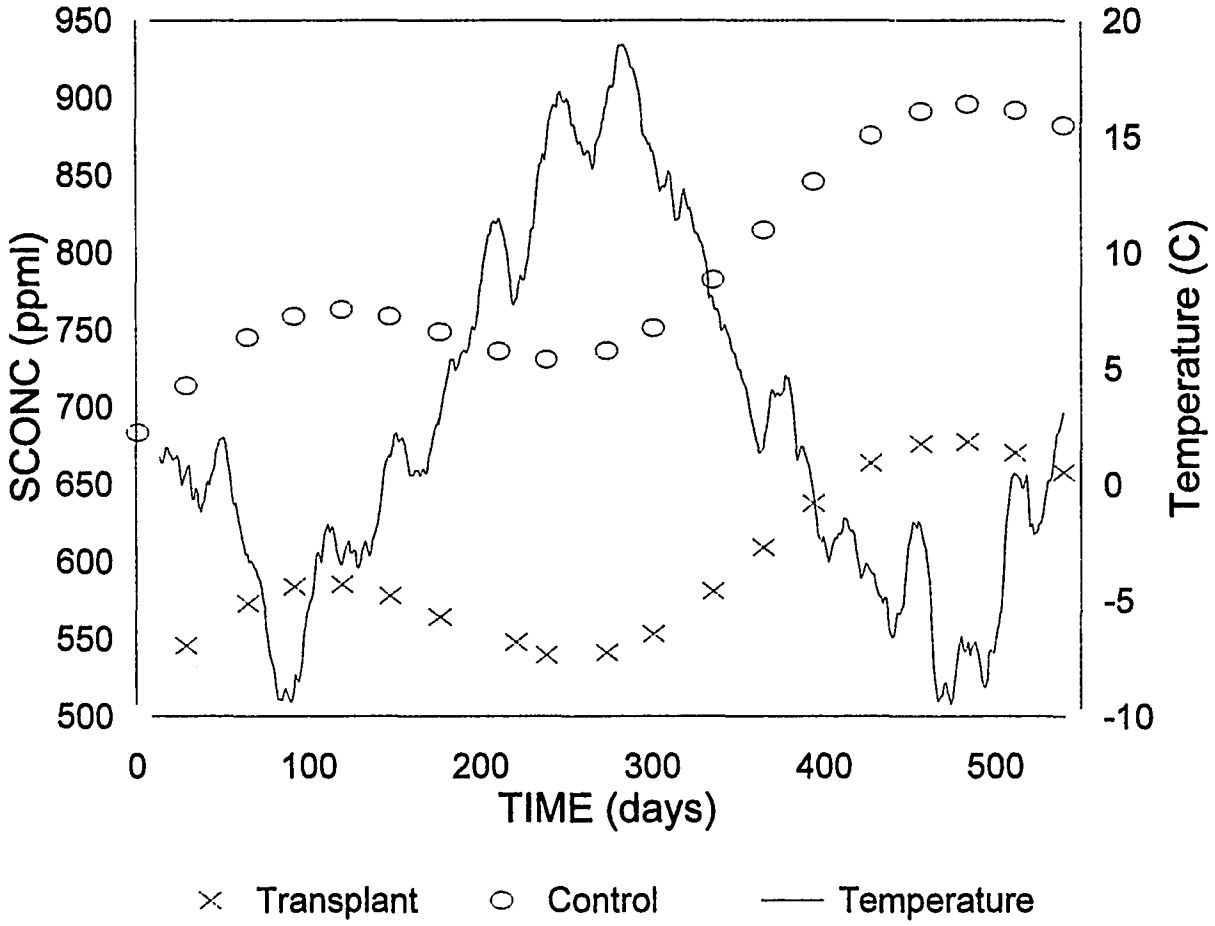



Figure 4.2: Regression model for sulphur concentration at the Botanical Gardens (Table 3.12) and mean daily temperature (degrees C; 15-day running mean).

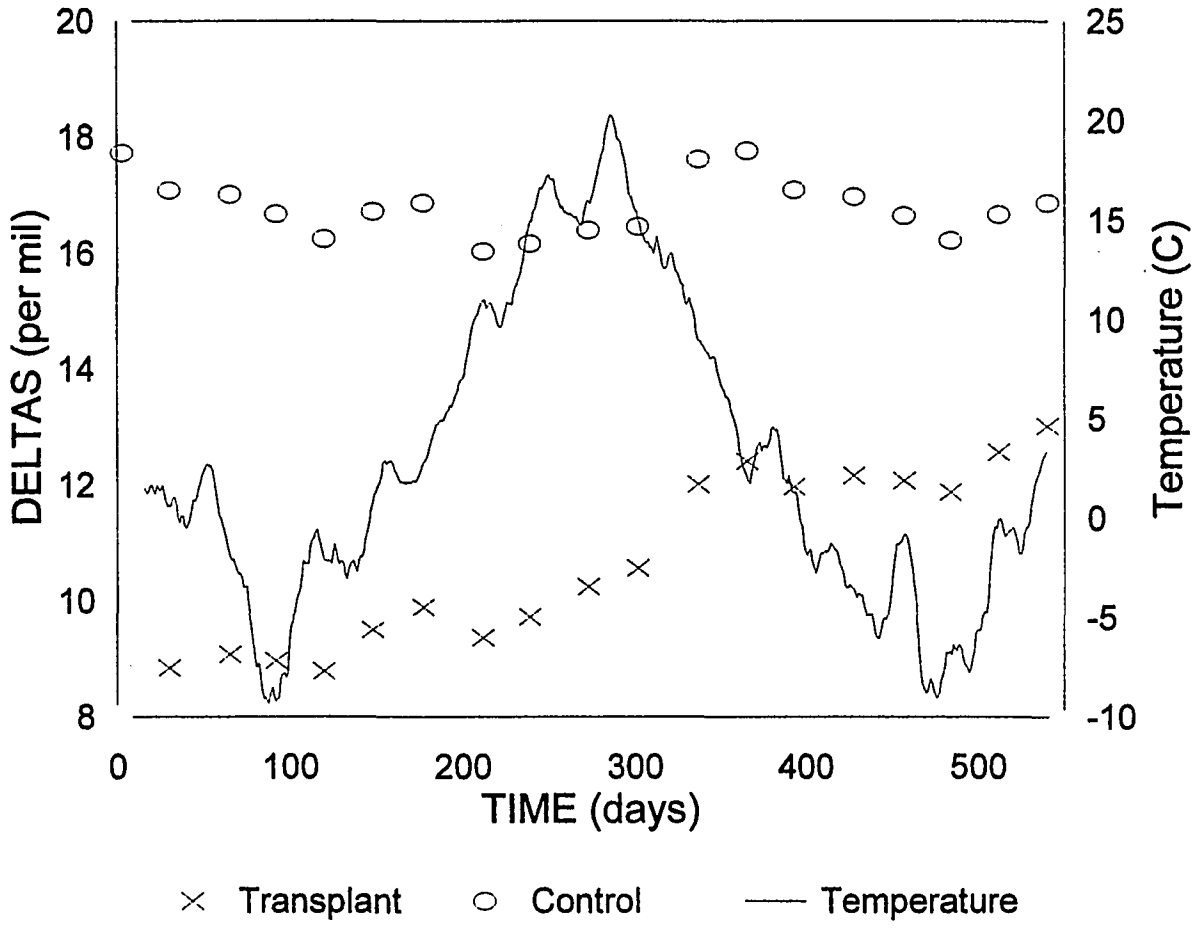



Figure 4.3: Regression model for sulphur isotopic composition at Riverhead (Table 3.14) and mean daily temperature (degrees C; 15-day running mean).

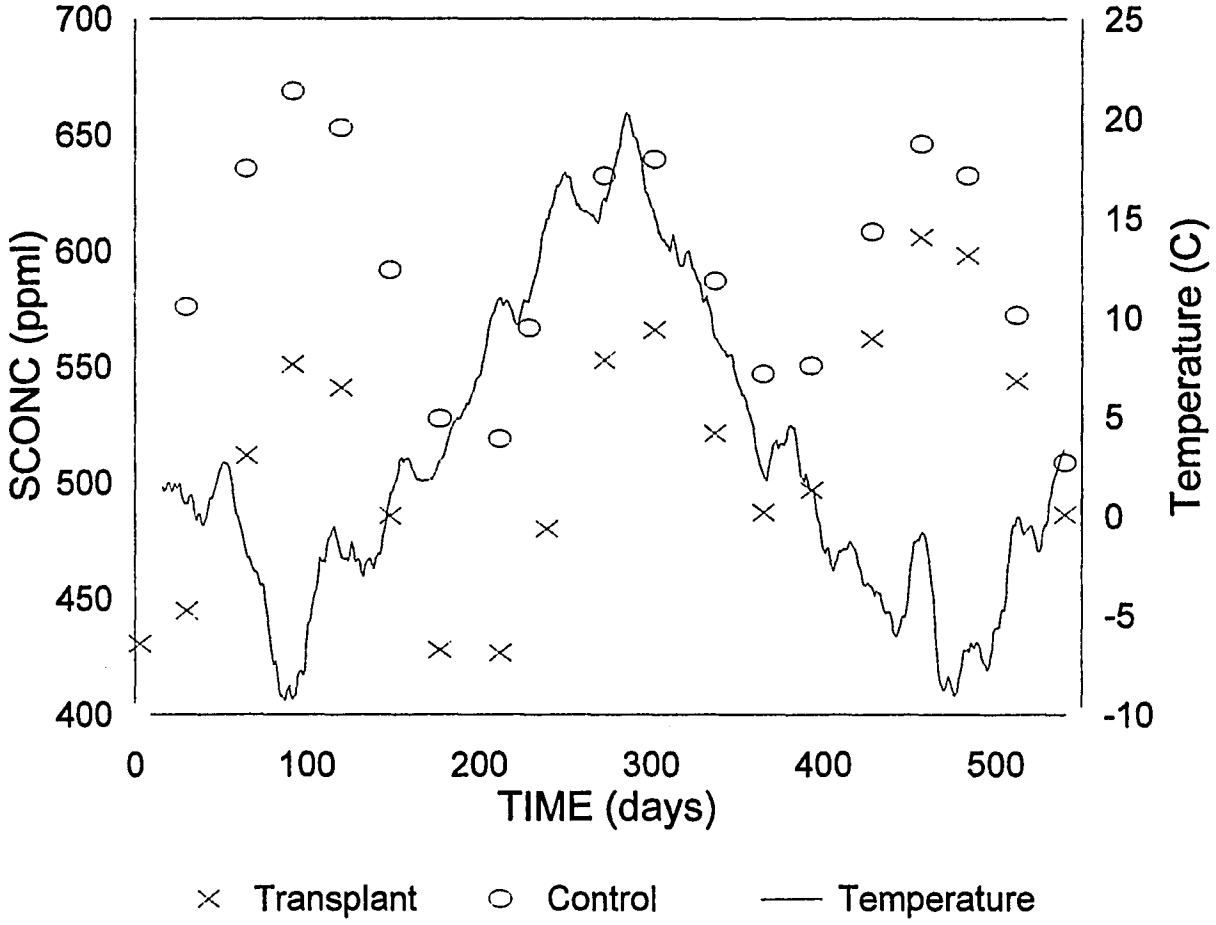



Figure 4.4: Regression model for sulphur concentration at Riverhead (Table 3.16) and mean daily temperature (degrees C; 15-day running mean).

# Chapter 5: Summary and Conclusions

## 5.1 Overview

The foremost objective of this experiment was to investigate the response of the epiphytic lichen *Alectoria sarmentosa* to changes in atmospheric sulphur conditions. Lichen thalli were transplanted reciprocally between two sites showing a large disparity in sulphur conditions and sampled and analysed monthly for sulphur isotopic composition and concentration. Lichen thalli were also transplanted within the same site. The reference (undisturbed lichens) and locally transplanted thalli were sampled concomitantly with the lichens transplanted reciprocally between the two sites. Specific objectives of the study were: i) to determine whether the act of transplanting alters the lichen response; ii) to ascertain the extent of background variation in sulphur conditions, and iii) to compare response times for the two directions of transplant.

As expected, the mechanics of relocation did not affect lichen response to changes in sulphur conditions. The change in sulphur isotopic composition and concentration in lichens transplanted locally was not significantly different from the change in undisturbed thalli at the same site.

Sulphur isotopic composition and concentration measurements in control lichens clearly indicated a predominance of anthropogenic sulphur sources at the Botanical Garden (mean $\delta^{34}S$ in control lichens = 6.33 ± 1.1 ‰) and natural (sea spray sulphates) sources at Riverhead (mean $\delta^{34}S$ in control lichens = 16.8 ± 1.4 ‰), in agreement with results from Wadleigh and Blake (1999) and Wiseman (1999).

Multiple regression with periodic functions was used to model the observations and proved useful and appropriate. Models generated with this parametric method were compared to curves obtained via the non-parametric smoothing technique LOWESS and found to be very similar. The use of multiple regression with periodic functions and concomitant transplant and control observations enabled the detection of cyclicity in the sulphur isotopic composition and concentration data. This was an improvement from the results of Wiseman and Wadleigh (2002), who used linear regression to model sulphur isotopic composition and concentration observations in lichens transplanted from a relatively unpolluted area to the Botanical Garden.

On the one hand, lichens originating from Riverhead and transplanted to the Botanical Garden responded to the change in sulphur source. It was estimated that 20 and 15 additional months would have been necessary for the lichens to reach local isotopic compositions at the Botanical Garden and Riverhead, respectively. On the other hand, the lichens transplanted to the Riverhead site showed a gradual decrease in sulphur concentration, but 3 additional months would have been necessary for the lichens to become indistinguishable from local ones. Any response to the change in concentration for lichens transplanted to the Botanical Garden was overshadowed by a rise in ambient sulphur levels over the course of the experiment. These time estimates took into account cyclicity in the observations.

The lag observed between sulphur isotopic compositions measured in lichens and the corresponding temperature effects was between 1 - 2 months at both sites. This was

speculated to be related to the time necessary between the assimilation of sulphur and its subsequent emission by the lichen. It was suggested that the lag observed in the isotopic composition models was indicative of biologically-bound sulphur, as there was no lag in the correspondence of sulphur concentration measurements and temperature patterns.

Transplantation of lichens from polluted to unpolluted conditions revealed seasonal cycles that could not be completely explained. Biogenic sources of sulphur may explain some of the results, especially during the dry/warm summer period. Results suggested that sulphur can be purged relatively fast from the thallus when lichens are subjected to lower ambient sulphur concentrations, possibly as a result of preferred loss of inorganic sulphur. We proposed that lichens can reflect small changes in isotopic composition over the short-term, but that much longer time-periods are required for larger disparities.

Isotope composition measurements provided more predictive power in the modelling of lichen responses, in addition to enabling to distinguish different sulphur sources.

## 5.2 Future research

Sulphur isotope composition and concentration measurements in lichens provide useful information on the response of lichens to changes in sulphur conditions. More research is needed with regard to the response of lichens to *improvements* in sulphur conditions. The results of this study have implications for monitoring studies, as they showed that relatively long periods of time (up to four years) may be necessary before

sulphur isotopic composition and concentration measurements in lichens reflect ambient conditions.

No distinction between the organic and inorganic fractions was made in this study. In many instances, phenomena observed during the course of this experiment may have been more easily explained had these two fractions been quantified. Additionally, measuring sulphur isotope composition and concentration in the apical regions of the thalli (newer portions) would undoubtedly have provided us with additional clues concerning mechanisms of sulphur accumulation and loss, and technological improvements may permit analysis of such small samples.

## References


AGUIAR, L.W., MARTAU, L., DE OLIVEIRA, M.A.A. and MARTINS-MAZZITELLI, S.M.A. 1998. Efeitos de dióxido de enxofre (SO2) em liquens, Rio Grande do Sul, Brasil. *Iheringia Serie Botanica*, 50: 67.

AHMADJIAN, V. 1967. The lichen symbiosis. Blaisdell Publishing Company, Waltham (Massachusetts). 152 pp.

AHTI, T. 1983. Lichens. pp. 319-360 *In* SOUTH, R. (Ed.). Biogeography and ecology of the island of Newfoundland. Dr. W. Junk Publishers, Den Haag. 723 pp.

ANDREAE, M.O. and BARNARD, W.R. 1984. The marine chemistry of dimethylsulfide. *Marine Chemistry*, 14: 267.

ANDREAE, M.O. and JAESCHKE, W.A. 1992. Exchange of sulphur between biosphere and atmosphere over temperate and tropical regions. pp. 27-66 *In* HOWARTH, R.W., STEWART, J.W.B. and IVANOV, M.V. (Eds). Sulphur cycling on the continents: wetlands, terrestrial ecosystems and associated water bodies. SCOPE 48. John Wiley and Sons, Chichester. 350 pp.

ANEJA, V.P. and COOPER, W.J. 1989. Biogenic sulfur emissions: a review. pp. 2-13 *In* SALTZMANN, E.S. and COOPER, W.J. (Eds). Biogenic sulfur in the environment. ACS Symposium Series 393. American Chemical Society, Washington, DC. 572 pp.

ANEJA, V.P., OVERTON, H.H., Jr., CUPITT, L.T., DURHAM, J.L.,and WILSON, W.E.. 1979. Carbon disulphide and carbonyl sulphide from biogenic sources and their contributions to the global sulphur cycle. *Nature*, 282: 493.

BALAGUER, L. and MANRIQUE, E. 1991. Interaction between sulfur dioxide and nitrate in some lichens. *Environmental and Experimental Botany*, 31 (2): 223.

BALAGUER, L., MANRIQUE, E. and ASCASO, C. 1997. Predictability of the combined effects of sulphur dioxide and nitrate on the green-algal lichen *Ramalina farinacea*. *Canadian Journal of Botany*, 75: 1836.

BEAUDOIN, G., TAYLOR, B.E., RUMBLE III, D., and THIEMENS, M. 1994. Variations in the sulfur isotope composition of troilite from the Cañon Diablo iron meteorite. *Geochimica et Cosmochimica Acta*, 56: 2839.

BERGMANN, L. and RENNENBERG, H. 1993. pp. 109-123 *In* DE KOK, LJ., STULEN, I., RENNENBERG, H., BRUNOLD, C. and RAUSER, W.E. (Eds). Sulfur nutrition and assimilation in higher plants. SPB Academic Publishing, The Hague.

BHATIA, S.P. 1978. Organosulfur emissions from industrial sources. pp. 51-83 *In* NRIAGU, J.O. (Ed.). Sulfur in the environment. Part 1: The atmospheric cycle. Wiley, New York. 464 pp.

BLAIR, G.J. 1979. Sulphur in the tropics. International Fertilizer Development Centre, Muscle Shoals, Alabama. 69 pp.

BLUM, O.B. 1973. Water relations. pp. 381-400 *In* AHMADJIAN, V. and HALE, M.E. (Eds). The lichens. Academic Press, New York. 697 pp.

BOERTJE, R.D. 1984. Seasonal diets of the Denali caribou herd, Alaska. *Arctic*, 37 (2): 161.

BRASSEUR, G.P., ORLANDO, J.J. and TYNDALL, G.S. (Eds.). 1999. Atmospheric chemistry and global change. Oxford University Press, New York. 654 pp.

BRIMBLECOMBE, P., HAMMER, C., RODHE, H., RYABOSHAPKO, A. and BOUTRON, C.F. 1989. Human influence on the Sulphur Cycle. pp. 77-121 *In* BRIMBLECOMBE, P. and LEIN, A.YU. Evolution of the global biogeochemical sulphur cycle. SCOPE 39. John Wiley and Sons, Chichester. 241 pp.

BRODO, I.M. 1961. Transplant experiments with corticolous lichens using a new technique. *Ecology*, 42: 838.

BRODO, I.M. and HAWKSWORTH, D.L. 1977. Alectoria and allied genera in North America. Opera Botanica, monograph No. 42. The Lund Botanical Society, Stockholm. 164 pp.

BRODO, I.M., SHARNOFF, S.D. and SHARNOFF, S. 2001. Lichens of North America. Yale University Press, New Haven and London. 795 pp.

BROWN, D.H. and BECKETT, R.P. 1984. Uptake and effect of cations on lichen metabolism. *Lichenologist*, 16: 173.

BROWN, D.H. and BROWN, R.M. 1990. Factors influencing dessication-induced membrane damage in *Peltigera horizontalis*. *Bibliotheca Lichenologica*, 38: 37.

BROWN, D.H., RAPSCH, S., BECKETT, A. and ASCASO, C. 1987. The effect of dessication on cell shape in the lichen *Parmelia sulcata* Taylor. *New Phytologist*, 105: 295.

BRUNOLD, C. 1990. Reduction of sulfate to sulfide. pp. 13-31 *In* RENNENBERG, H., BRUNOLD, C., DE KOK, L.J. and STULEN, I. (Eds.). Sulfur nutrition and sulfur assimilation in higher plants. SPB Academic Publishing, Den Haag.

BÜDEL, B. 1987. Zur Biologie und Systematik der Flechtengattungen *Heppia* und *Peltula* im südlichen Afrika. *Bibliotheca Lichenologica*, 23: 1-105.

BÜDEL, B. and LANGE. O.L. 1991. Water status of green and blue-green phycobionts in lichen thalli after hydration by water vapor uptake: do they become turgid? *Botanica Acta*, 104: 361.

BÜDEL, B. and SCHEIDEGGER, C. 1996. Thallus morphology and anatomy. *In* NASH III, T.H. (Ed.). pp. 37-64. Lichen Biology. Cambridge University Press, Cambridge. 303 pp.

CARRERAS, H.A. and PIGNATA, M.L. 2001. Comparison among air pollutants, meteorological conditions and some chemical parameters in the transplanted lichen *Usnea amblyoclada*. *Environmental Pollution*, 111: 45.

CASE, J.W. and KROUSE, H.R. 1980. Variations in sulphur content and stable isotope composition of vegetation near a $SO_2$ source at Fox Creek, Alberta, Canada. *Oecologia* (Berlin), 44: 248.

CHARLSON, R.J., LOVELOCK, J.E., ANDREAE, M.O. and WARREN, S.G. 1987. Oceanic phytoplankton, atmospheric sulphur, cloud albedo and climate. *Nature*, 326: 655.

CLEVELAND, W.S. 1979. Robust locally weighted regression and smoothing scatterplots. *Journal of the American Statistical Association*, 74: 829.

COLLINS, C.R. and FARRAR, J.F. 1978. Structural resistances to mass transfer in the lichen *Xanthoria parietina*. *New Phytologist*, 31: 71.

COX, R.A. and SHEPPARD, D. 1980. Reactions of OH radicals with gaseous sulphur compounds. *Nature* (London), 284: 330.

CRÊTE, M., HUOT, J. and GAUTHIER, L. 1990. Food selection during early lactation by caribou calving on the toundra in Québec. *Arctic*, 43:60.

DÄSSLER, H.G. and RANFT, H. 1969. Flora, 158: 454

DECORMIS, L. 1969. Dégagement d'hydrogène sulfure par des plantes soumises à une atmosphère contenant de l'anhydride sulfureux. *Comptes Rendus de L'Académie des Sciences, Série D*, 266: 683.

DE KOK, L.J. 1990. Sulfur metabolism in plants exposed to atmospheric sulfur. pp. 111-130 *In* DE KOK, LJ., STULEN, I., RENNENBERG, H., BRUNOLD, C. and RAUSER, W.E. (Eds). Sulfur nutrition and assimilation in higher plants. SPB Academic Publishing, The Hague.

DELTORO, V.I., GIMENO, C., CALATAYUD, A. and BARRENO, E. 1999. Effects of $SO_2$ on photosynthetic $CO_2$ gas exchange, chlorophyll a fluorescence emission and antioxidant enzymes in the lichens *Evernia prunastri* and *Ramalina farinacea*. *Physiologia Plantarum*, 105: 648.

ENNIS, L. 1999. Isotopic composition of Bunker C fuels from major anthropogenic sulphur sources in Newfoundland. B.Sc.(Hon.) Dissertation, Memorial University of Newfoundland. 49 pp.

EPSTEIN, E., SAGEE, O., COHEN, J.D. and GARTY, J. 1986. Endogenous auxin and ethylene in the lichen *Ramalina duriaei*. *Plant Physiology*, 82: 1122.

EVERSMAN, S. and SIGAL, L.L. 1987. Effects of $SO_2$, $O_3$ and $SO_2$ and $O_3$ in combination on photosynthesis and ultrastructure of two lichen species. *Canadian Journal of Botany*, 65: 1806.

FARRAR, J.F. 1976. The uptake and metabolism of phosphate by the lichen *Hypogymnia physodes New Phytologist*, 77: 127.

FEREK, R.J., CHATFIELD, T.B. and ANDREAE, M.O. 1986. Vertical distribution of dimethylsulphide in the marine atmosphere. *Nature*, 307: 148.

FIELDS, R.F. 1988. Physiological responses of lichens to air pollutant fumigations. pp. 175-200. *In* NASH III, T.H. and WIRTH, V. (Eds). Lichens, bryophytes and air quality. Bibliotheca Lichenologica, 30. J. Cramer, Berlin-Stuttgart.

FIELDS, R.D. and St. CLAIR, L.L. 1984. The effects of $SO_2$ on photosynthesis and carbohydrate transfer in the two lichens: *Collema polycarpon* and *Parmelia chlorochroa*. *American Journal of Botany*, 71 (7): 986.

GAILEY, F.A.Y., SMITH, G.H., RINTOUL, L.J. and LLOYD, O.L. 1985. Metal deposition patterns in central Scotland, as determined by lichen transplants. *Environmental Monitoring and Assessment*, 5: 291.

GALUN, M. and RONEN, R. 1988. Interactions of lichens and pollutants. pp. 55-73 *In* GALUN, M. (Ed). Handbook of lichenology. Volume 3. CRC Press, Boca Raton. 147 pp.

GARTY, J., COHEN, Y. and KLOOG, N. 1998a. Airborne elements, cell membranes, and chlorophyll in transplanted lichens. *Journal of Environmental Quality*, 27 (4): 973.

GARTY, J., FUCHS, C. ZISAPEL, N. and GALUN, M. 1977. Heavy metals in the lichen *Caloplaca aurantia* from urban, suburban and rural regions in Israel (a comparative study). *Water, Air and Soil Pollution*, 8: 171.

GARTY, J., GALUN, M. and KESSEL, M. 1979. Localization of heavy metals and other elements accumulated in the lichen thallus. *New Phytologist*, 82: 159.

GARTY, J., KARARY, Y., and HAREL, J. 1992. Effect of low pH, heavy metals and anions on chlorophyll degradation in the lichen *Ramalina duriaei* (De Not.) Bagl. *Environmental and Experimental Botany*, 32 (3): 229.

GARTY, J., KARARY, Y., HAREL, J. and LURIE, S. 1993. Temporal and spatial fluctuations of ethylene production and concentrations of sulfur, sodium, chlorine, and iron on/in the thallus cortex in the lichen *Ramalina duriaei* (De Not.) Bagl. *Environmental and Experimental Botany*, 33(4): 553.

GARTY, J., KAUPPI, M. and KAUPPI, A. 1996. Accumulation of air elements from vehicles in transplanted lichen in urban sites. *Journal of Environmental Quality*, 25: 265.

GARTY, J., KAUPPI, M. and KAUPPI, A. 1997a. The influence of air pollution on the concentration of airborne elements and on the production of stress-ethylene in the lichen *Usnea hirta* (L.) Weber em. Mot. transplanted in urban sites in Oulu, N. Finland. *Archives of Environmental Contamination and Toxicology*, 32: 285.

GARTY, J., KLOOG, N. and COHEN, Y. 1998b. Integrity of lichen cell membranes in relation to concentration of airborne elements. *Archives of Environmental Contamination and Toxicology*, 34: 136.

GARTY, J., KLOOG, N., COHEN, Y., WOLFSON, R. and KARNIELI, A. 1997b. The effect of air pollution on the integrity of chlorophyll, spectral reflectance response, and on concentrations of nickel, vanadium, and sulfur in the lichen *Ramalina duriaei* (De Not.) Bagl. *Environmental Research*, 74: 174.

GARTY, J., KLOOG, N., WOLFSON, R., COHEN, Y., KARNIELI, A. and AVNI, A. 1997c. The influence of air pollution on the concentration of mineral elements, on the spectral reflectance response and on the production of stress-ethylene in the lichen *Ramalina duriaei. The New Phytologist*, 137 (4):587.

GARTY, J. RONEN, R. GALUN, M. 1985. Correlation between chlorophyll degradation and the amount of some elements in the lichen *Ramalina duriaei* (De Not.) Jatta. *Environmental and Experimental Botany*, 25: 67

GARTY, J., WEISSMAN, L., COHEN, Y., KARNIELI, A. and ORLOVSKY, L. 2001. Transplanted lichens in an around the Mount Carmel National Park and the Haifa Bay industrial region in Israel: physiological and chemical responses. *Environmental Research*, 85A: 159.

GAUTHIER, L., NAULT, R. and CRÊTE, M. 1989. Variations saisonnières du régime alimentaire des caribous du nord du Québec. *Nature Canadienne*, 116: 101.

GILBERT, O.L. 1973. Lichens and air pollution. pp. 443-472 *In* AHMADJIAN, V. and HALE, M.E. (Eds). The Lichens. Academic Press, New York. 697 pp.

GLASS, S.J. and MATTESON, M.J. 1973. Ion enrichment in aerosols dispersed from bursting bubbles in aqueous salt solutions. *Tellus*, 25: 272.

GOLDAN, P.D., KUSTER, W.C., ALBRITTON, D.L., and FEHSENFELD, F.C. 1987. The measurement of natural sulfur emissions from soils and vegetation: Three sites in the Eastern United States revisited. *Journal of Atmospheric Chemistry*, 5: 439.

GONZÁLEZ, C., CASANOVAS, S.S. and PIGNATA, M.L. 1996. Biomonitoring of air pollutants from traffic and industries employing *Ramalina ecklonii* (Spreng.) Mey and Flot. *Environmental Pollution*, 91: 269.

GONZÁLEZ, C., ORELLANA, L.C., CASANOVAS, S.S. and PIGNATA, M.L. 1998. Environmental conditions and chemical response of a transplanted lichen to an urban area. *Journal of Environmental Management*, 53: 73.

GONZÁLEZ, C. and PIGNATA, M.L. 1994. The influence of air pollution on soluble proteins, chlorophyll degradation, MDA, sulphur and heavy metals in a transplanted lichen. *Chemistry and Ecology*, 9: 105.

GONZÁLEZ, C. and PIGNATA, M.L. 1997. Chemical response of the lichen *Punctelia subrudecta* (Nyl.) Krog transplanted close to a power station in an urban-industrial environment. *Environmental Pollution*, 97 (3): 195.

GONZÁLEZ, C. and PIGNATA, M.L. 2000. Chemical response of transplanted lichen *Canomaculina pilosa* to different emission sources of air pollutants. *Environmental Pollution*, 110: 235.

GORIN, P.A.J., BARON, M. and IACOMINI, M. 1988. Storage products of lichens. pp. 9-24 *In* GALUN, M. (Ed). Handbook of lichenology. Volume 2. CRC Press, Boca Raton. 147 pp.

GRINENKO, V.A. and IVANOV, M.V. 1983. Principal reactions of the global biogeochemical cycle of sulphur. pp. 1-23 In IVANOV, M.V. and FRENEY, J.R. (Eds. ). The global biogeochemical sulphur cycle. SCOPE 19. John Wiley and Sons, Chichester. 470 pp.

HÄFFNER, E., LOMSKÝ, B., HYNEK, V., HÄLLGREN, J.E., BATIČ, F. and PFANZ, H. 2001. Physiological responses of different lichens in a transplant experiment following and $SO_2$-gradient. *Water, Air and Soil Pollution*, 131: 185.

HALE, M.E. Jr. 1974. The Biology of Lichens. 2nd Edition. Edward Arnold Ltd., London. 181 pp.

HELSEL, D.R. and HIRSCH, R.M. 1992. Statistical methods in water resources. Studies in Environmental Science 49. Elsevier Science, Amsterdam. 522 pages.

HIRSCH, R.M., SLACK, J.R., and SMITH, R.A. 1982. Techniques of trend analysis for monthly water quality data. *Water Resources Research*, 18: 107.

HENRIKSSON, E. and PEARSON, L.C. 1981. Nitrogen fixation rate and chlorophyll content of lichen *Peltigera canina* exposed to sulphur dioxide. *American Journal of Botany*, 68: 680.

HILL, F.B., ANEJA, V.P., and FELDER, R.M. 1978. A technique for measurement of biogenic emission fluxes. *Journal of Environmental Science and Health*, 13: 199.

HOEFS, J. 1987. Stable isotope geochemistry. 3rd Edition. Springer, Berlin. 201 pp.

HOLLAND, H.D. 1978. The chemistry of the atmosphere and oceans. Wiley, New York. 351 pp.

HOLSER, W.T., MAYNARD, J.B. and CRUIKSHANK, K.M. 1989. Modelling the natural cycle of sulphur through phanerozoic time. pp. 21-56 *In* BRIMBLECOMBE, P. and LEIN, A.YU. Evolution of the global biogeochemical sulphur cycle. SCOPE 39. John Wiley and Sons, Chichester. 241 pp.

HONNEGER, R. 1991. Functional aspects of the lichen symbiosis. *Annual Review of Plant Physiology and Plant Molecular Biology*, 42: 553.

HONNEGER, R. 1996. Morphogenesis. pp. 65-87 *In* NASH III, T.H. (Ed.) 1996. Lichen Biology. Cambridge University Press, Cambridge. 303 pp.

HOPP, U. and KAPPEN, L. 1981. Einige Aspekte zur Immissionbedingten Verbreitung von Flechten im Stadtgebiet von Würzburg. *Berichte. Bayerische Botanische Gesellschaft.*, 52: 15.

INGVORSEN, K. and JORGENSEN, B.B. 1982. *Atmospheric Environment*, 16: 855-865.

JAHNS, H.M. 1988. The lichen thallus. pp. 95-158 *In* GALUN, M. (Ed.). Handbook of lichenology. Volume 1. CRC Press, Boca Raton. 297 pp.

JAMIESON, R. 1996. A stable isotope study of natural and anthropogenic sulphur in precipitation in Eastern Canada. M.Sc. Dissertation, Memorial University of Newfoundland. 183 pp.

JAMIESON, R.E. and WADLEIGH, M.A. 1999. A Study of the oxygen isotopic composition of precipitation sulphate in eastern Newfoundland. *Water, Air and Soil Pollution*, 110: 405.

JAMIESION, R.E. and WADLEIGH, M.A. 2000. Tracing sources of precipitation sulfate in eastern Canada using stable isotopes and trace metals. *Journal of Geophysical Research*, 105 (D16): 20 549.

JOHNSEN, I.B., 1981. Heavy metal deposition on plant in relation to immission and bulk precipitation. *Silva fennica*, 15: 444.

JONES, D., WILSON, M.J. and LAUNDON, J.R. 1982. Observations on the location and form of lead in *Stereocaulon vesuvianum. Lichenologist*, 14: 281.

KANTVILAS, G., JAMES, P.W. AND JARMAN, S.J. 1985. Macrolichens in Tasmanian rainforests. *Lichenologist*, 17: 67.

KAUPPI, M., KAUPPI, A. and GARTY, J. 1998. Ethylene produced by the lichen *Cladina stellaris* exposed to sulphur and heavy-metal-containing solutions under acidic conditions. *New Phytologist*, 139 (3): 537.

KELLOGG, W.W., CADLE, R.D., ALLEN, E.R., LAZRUS, A.L. and MARTELL, E.A. 1972. The sulphur cycle. *Science* (Washington D.C.), 175: 587.

KERSHAW, K.A. 1985. Physiological ecology of lichens. Cambridge University Press, Cambridge. 293 pp.

KIYOURA, R., KURONUMA, H. UWANISHI, G. *et al.* 1970. Some opinions on sulphur dioxide as an atmospheric pollutant. *Bulletin of the Tokyo Institute of Technology*, 98: 117.

KNOPS, J.M.H., NASH III, T.H., BOUCHER, V.L. and SCHLESINGER, W.H. 1991. Mineral cycling and epiphytic lichens: implications at the ecosystem level. *Lichenologist*, 23: 309.

KONG, F.X., HU, W., CHAO, S.Y., SANG, W.L. and WANG, L.S. 1999. Physiological responses of the lichen *Xanthoparmelia mexicana* to oxidative stress of $SO_2$. *Environmental and Experimental Botany*, 42: 201.

KRANNER, I. and GRILL, D. 1997. Dessication of lichens: changes in the glutathione status. pp. 253-255 *In* CRAM, W.J., DE KOK, L.J., STULEN, I., BRUNOLD, C. and RENNENBERG, H. Sulphur metabolism in higher plants. CRAM, W.J., DE KOK, L.J., STULEN, I., BRUNOLD, C. and RENNENBERG, H. Backhuys Publishers, Leiden. 367 pp.

KROUSE, H.R. 1977. Sulphur isotope abundance elucidate uptake of atmospheric sulphur emissions by vegetation. *Nature*, 265: 45.

KROUSE, H.R., LEGGE, A.H. and BROWN, H.M. 1984. Sulphur gas emission in the boreal forest: the West Whitecourt case study - V. Stable sulphur isotopes. *Water, Air, and Soil Pollution*, 22: 321.

KROUSE, H.R., STEWART, J.W.B. and GRINENKO, V.A. 1991. Pedosphere and Biosphere. pp. 267-306 *In* KROUSE, H.R. and GRINENKO, V.A. (Eds.) Stable isotopes: natural and anthropogenic sulphur in the environment. SCOPE 43. John Wiley and Sons, Chichester. 440 pp.

KROUSE, H.R. and VAN EVERDINGEN, R.O. 1984. $\delta^{34}S$ variations in vegetation and soil exposed to intense biogenic sulphide emissions near Paige Mountain, N.W.T., Canada. *Water, Air and Soil Pollution*, 23: 61.

LANGE, O.L. 1953. Hitze- und Trockenresistenz der Flechten in Beziehung zu ihrer Verbreitung. *Flora (Jena)*, 140: 39.

LANGE, O.L., HEBER, U., SCHULZE, E.D. and ZIEGLER, H. 1989. Atmospheric pollutants and plant metabolism. *Ecological Studies*, 7: 238.

LEBLANC, F. and DE SLOOVER, J. 1970. Relation between industrialization and the distribution and growth of epiphytic lichens and mosses in Montreal. *Canadian Journal of Botany*, 48: 1485.

LEBLANC, R., RAO, D.N. and COMEAU, G. 1972. Indices of atmospheric purity and fluoride pollution pattern in Arvida, Québec. *Canadian Journal of Botany*, 50: 991.

LEVIN, A.G. and PIGNATA, M.L. 1995. *Ramalina ecklonii* as bioindicator of atmospheric pollution in Argentina. *Canadian Journal of Botany*, 73: 1196.

LINK, S.O. and NASH, T.H. 1984. Ecophysiological studies of the lichen *Parmelia praesignis* Nyl.: population variation and the effects of storage conditions. *New Phytologist*, 96: 249.

MACINTYRE, F. 1974. The top millimeter of the ocean. *Scientific American*, 230 (5): 62.

MÁGUAS, C. and BRUGNOLI, E. 1996. Spatial variation in carbon isotope discrimination across the thalli of several lichen species. *Cell and Environment*, 19:437.

MÁGUAS, C., GRIFFITHS, H., EHLERINGER, J. and SERÔDIO, J. 1993. Characterization of photobiont associations in lichens using carbon isotope discrimination techniques. pp. 201-212 *In* EHLERINGER, J.E., HALL, A.E. and FARQUHAR, G.D. (Eds).

Stable isotopes and plant carbon-water relations. Academic Press Inc., San Diego. 555 pp.

MALHOTRA, S.S. and KHAN, A.A. 1983. Sensitivity to $SO_2$ of various metabolic processes in an epiphytic lichen, *Evernia mesomorpha. Biochemie und Physiologie der Pflanzen*, 178: 121.

MAYNARD, D.G. STEWARD J.W.B. and BETTANY, J.R. 1984. Sulfur cycling in grassland and parkland soils. *Biogeochemistry*, 1: 97.

MEISTER, A. 1995. *Methods Enzymol.* 251: 3.

MEKHTIYEVA, V.L. 1971. Isotopic composition of sulphur from plants and animals in water basins of various salinities. *Geokhimiya*, 6: 725.

MENZEL, D.B. 1976. The role of free radicals in the toxidicty of air pollutants (nitrogen oxides and ozone). pp. 181-203 *In* W.A. PRYOR (Ed.). Free Radicals in Biology. Vol. 2. Academic Press, New York. 303 pp.

MOSER, T.J., NASH, T.H.III, OLAFSEN, A.G. 1983. Photosynthetic recovery in arctic caribou forage lichens following a long-term field sulfur dioxide fumigation. *Canadian Journal of Botany*, 61: 367.

MOSS, M.R. 1978. Sources of sulfur in the environment: the global sulfur cycle. p. 23-50 *In* NRIAGU, J.O. (Ed.). Sulfur in the Environment. Part I: The Atmospheric Cycle. John Wiley and Sons, New York. 464 pp.

NASH III, T.H. (Ed.) 1996. Lichen Biology. Cambridge University Press, Cambridge. 303 pp.

NEWMAN, L. , KROUSE, H.R. and GRINENKO, V.A. 1991. Sulphur isotope variations in the atmosphere. pp. 133-176 *In* KROUSE, H.R. and GRINENKO, V.A. (Eds.) Stable isotopes: natural and anthropogenic sulphur in the environment. SCOPE 43. John Wiley and Sons, Chichester. 440 pp.

NIEBOER, E. and RICHARDSON, D.H.S. 1980. The replacement of the nondescript term 'heavy metals' by a biologically and chemically significant classification of metal ions. *Environmental Pollution*, 1: 3.

NIELSEN, H. 1974. Isotopic composition of the major contributors to atmospheric sulphur. *Tellus*, 26 (1-2):213.

NRIAGU, J.O. and COKER, R.D. 1978. Isotopic composition of sulphur in precipitation within the Great Lakes basin. *Tellus*, 30: 365.

NYLANDER, W. 1866. Les lichens du jardins du Luxembourg. *Bulletin de la Société Botanique de France*, Séance du 13 Juillet 1866: 364.

PALOMÄKI, V., TYNNYRINEN, S. and HOLOPAINEN, T. 1992. Lichen transplantation in monitoring fluoride and sulphur deposition in the surroundings of a fertilizer plant and a strip mine at Siilinjärvi. *Annale Botanica Fennici*, 29: 25.

PAWLIK-SKOWROSKA, B., SANITÁ DI TOPPI, L., FAVALI, M.A., FOSSATI, F. and SKOWRO SKI, T. 2001. Response of lichens to heavy metal stress by phytochelatin production. *Acta Physiologiae Plantarum*, 23 (3): 80.

PEARSON, L.C. and HENRIKSSON, E. 1981. Air pollution damage to cell membranes in lichens II. Laboratory experiments. *The Bryologist*, 4:515.

PEARSON, L.C. and RODGERS, G.A. 1982. Air pollution damage to cell membranes in lichens III. Field experiments. *Phyton* (Austria), 22:329.

PERKINS, D.F. and MILLAR, R.O. 1987a. Effects of airborne fluoride emissions near an aluminum works in Wales: Part 1 - Corticolous lichens growing on broadleaved trees. *Environmental Pollution*, 47: 63.

PERKINS, D.F. and MILLAR, R.O. 1987b. Effects of airborne fluoride emissions near an aluminum works in Wales: Part 2 - Saxicolous lichens growing on rocks and walls. *Environmental Pollution*, 48: 185.

PILEGAARD, K. 1979. Heavy metals in bulk precipitation and transplanted *Hypogymnia physodes* and *Dicranoweisia cirrata* in the vicinity of a Danish steelworks. *Water, Air and Soil Pollution*, 11: 77.

PUCKETT, K.J. 1988. Bryophytes and lichens as monitors of metal deposition. p. 231-367 *In* NASH III, T.H. and WIRTH, V. (Eds). Lichens, bryophytes and air quality. Bibliotheca Lichenologica, 30. J. Cramer, Berlin-Stuttgart.

PUCKETT, K.J. and FINEMAN, E.J. 1980. An analysis of the element content of lichens from the Northwest Territories, Canada. Canadian Journal of Botany, 58: 2073.

PUCKETT, K.J., RICHARDSON, D.H.S., FLORA, W.P. and NEIBOER, E. 1974. Photosynthetic $^{14}C$ fixation by the lichen *Umbilicaria muhlenbergii* (Ach.) Tuck. following short exposure to aqueous sulfur dioxide. *The New Phytologist*, 73: 1183.

PURVIS, O.W., WILLIAMSON, B.J., BARTOK, K. and ZOLTANI, N. 2000. Bioaccumulation of lead by the lichen *Acaspora smaragdula* from smelter emissions. *The New Phytologist*, 147 (3) :591.

RALPH, B.J. 1979. Oxidative reactions in the sulfur cycle. pp. 369-400. *In* TRUDINGER, P.A. and SWAINE, D.J. (Eds). Biogeochemical cycling of mineral-forming elements. Elsevier Scientific, Amsterdam. 612 pp.

REES, C.E., JENKINS, W.J. and MONSTER, J. 1978. The sulphur isotopic composition of ocean water sulphate. *Geochimica et Cosmochimica Acta*, 42: 377.

REIS, M.A., ALVES, L.C., FREITAS, M.C., VAN OS, B., DE GOEIJ, J.and WOLTERBEEK, H.TH. 2002. Calibration of lichen transplants considering faint memory effects. *Environmental Pollution*, 120: 87.

REUTIMANN, P. and SCHEIDEGGER, C. 1987. Importance of lichen secondary products in food choice of two oribatic mites (Acari) in an alpine meadow ecosystem. *Journals of Chemical Ecology*, 13: 363.

RICHARDSON, D.H.S. 1988. Understanding the pollution sensitivity of lichens. *Botanical Journal of the Linnean Society*, 96: 31.

RICHARDSON, D.H.S. 1992. Pollution monitoring with lichens. The Richmond Publishing Co. Ltd., England. 76 pp.

RICHARDSON, D.H.S., KIANG, S., AHMADJIAN, V. and NIEBOER, E. 1985. Lead and uranium uptake by lichens. .pp. 227-246 *In* BROWN, D.H. (Ed.). Lichen Physiology and Cell Biology. Plenum Publishing, London. 362 pp.

RICHARDSON, D.H.S. and NIEBOER, E. 1981. Lichens and pollution monitoring. *Endeavour*, 5 (3): 127.

RICHARDSON, D.H.S. and NIEBOER, E. 1983. Ecological responses of lichens to sulphur dioxide. *Journal of the Hattori Botanical Laboratory*, 54: 331.

ROMINGER, E.M. and OLDEMEYER, J.L. 1990. Early-winter diet of woodland caribou in relation to snow accumulation. *Canadian Journal of Zoology*, 68: 2691.

ROUX, M. 1981. Étude écologique et phytosociologique des peuplements lichéniques saxicoles-calcicoles du sud-est de la France. *Bibliotheca Lichenologica*, 15: 1-557.

ROY, A.B. and TRUDINGER, P.A. 1970. The biochemistry of inorganic compounds of sulphur. Cambridge University Press, Cambridge. 400 pp.

RUNDEL, P.W. 1980. Corticolous lichen communities of Nothofagus dombeyi on Volcan Villarica in southern Chile. *Bryologist*, 83: 82.

RUNDEL, P.W. 1988. Water relations. pp. 17-36 *In* GALUN, M. (Ed). Handbook of lichenology. Volume 3. CRC Press, Boca Raton. 147 pp.

RYABOSHAPKO, A.G. 1983. The atmospheric sulphur cycle. pp. 203-296 *In* IVANOV, M.V. and FRENEY, J.R. (Eds). The global biogeochemical sulphur cycle. SCOPE 19. John Wiley and Sons, Chichester. 470 pp.

SCHIFF, J.A. 1983. Reduction and other metabolic reactions of sulfate. pp. 401-421 *In* LÄUCHLI, A. and BIELESKI, R.L. (Eds). Inorganic plant nutrition. Encyclopedia of plant physiology. New Series, Volume 15A.. Springer-Verlag, Berlin-Heidelberg. 449 pp.

SCHLESINGER, W. H. 1991. Biogeochemistry: an analysis of global change. Academic Press Inc., London. 443 pp.

SCHONBECK, H. and VAN HUT, H. 1971. Exposure of lichens for the recognition and the evaluation of air pollutants. pp. 329-334 *In* National Research Council of Canada. Identification and Measurement of Environmental Pollutants. Proceedings of the International Symposium, Ottawa, Ontario, June 1971.

SEITZ, W. 1972. Flechtenwuchs und Luftverunreinigung im Grossraum von Saarbrücken. *Berichte. Deutsche Botanische Gesellschaft*, 85:129.

SHOWMAN, R.E. and LONG, R.P. 1992. Lichen studies along a wet sulfate deposition gradient in Pennsylvania. *Bryologist*, 95 (2): 166.

SIGAL, L.L. and NASH, T.H. 1983. Lichen communities on conifers in southern California mountains: an ecological survey relative to oxidant air pollution. *Ecology*, 64: 1343.

SKYE, E. 1968. Lichens and air pollution. A study of cryptogamic epiphytes and environment in the Stockholm region. *Acta Phytogeographica Suecica*, 52: 8.

SKYE, E. and HALLBERG, I. 1969. Changes in the lichen flora following air pollution. *Oikos*, 20: 547.

SLOOF, J.E. 1995. Lichens as quantitative biomonitrors for atmospheric trace-element deposition, using transplants. *Atmospheric Environment*, 29 (1): 11.

SMITH, A.L. 1921. Lichens. Cambridge University Press, London. 464 pp.

SMITH, D.C. and MOLESWORTH, S. 1973. Lichen physiology. XIII. Effects of rewetting dry lichens. *New Phytologist*, 72: 525.

SPALENY, J. 1977. Sulphate transformation to hydrogen sulphide in spruce seedlings. *Plant and Soil*, 48: 557.

SUGIYAMA, K., KUROKAWA, S., and OKADAM, G. 1976. Studies on lichens as a bioindicator of air pollution. Correlation of distribution of *Parmelia tinctorum* with $SO_2$ air pollution. *Japanese Journal of Ecology*, 26:109.

TAKÁCS, Z., CSINTALAN, Z. and TUBA, Z. 1999. Responses of the lichen Cladonia convoluta to high $CO_2$ level and heavy metal treatment. *Zeitschrift für Naturforschung*, 54C: 797.

TAKALA, K., OLKKONEN, H., IKONEN, J., JÄÄSKELÄINEN, J, and PUUMALAINEN, P. 1985. Annale Botanica Fennici, 22: 91.

TAIZ, L. and ZEIGER, E. 1998. Plant physiology. Sinauer Associates, Sunderland, Massachusetts. 792 pp.

TAN, C. and LIU, Y. 1981. Research on the reaction and resistance of plants to $SO_2$. VIII. Protection of radical scavengers to impairment of $SO_2$. *Journal of Environmental Science*, 1:197.

TARHANEN, S., POIKOLAINEN, J., HOLOPAINEN, T. and OKSANEN, J. 2000. Severe photobion injuries of lichens are strongly associated with air pollution. *The New Phytologist*, 177 (3): 579.

TEERI, J.A. 1981. Stable carbon isotope analysis of mosses and lichens growing in xeric and moist habitats. *The Bryologist*, 84 (1): 82.

THODE, H.G. 1991. Sulphur isotopes in nature and the environment: an overview. pp. 1-26 *In* KROUSE, H.R. and GRINENKO, V.A. (Eds). Stable isotopes: natural and

anthropogenic sulphur in the environment. SCOPE 43. John Wiley and Sons, Chichester. 440 pp.

TOMASSINI, F.D., LAVOIE, K.J., PUCKETT, E., NIEBOER, E. and RICHARDSON, D.H.S. 1977. The effect of time of exposure to sulfur dioxide on potassium loss and photosynthesis in the lichen *Cladonia rangiformis* (L.) Harm. *New Phytologist*, 79:147.

TYLER, G. 1989. Uptake, retention and toxicity of heavy metals in lichens. *Water, Air and Soil Pollution*, 47: 321.

WADLEIGH, M.A. and BLAKE, D.M. 1999. Tracing sources of atmospheric sulphur using epiphytic lichens. *Environmental Pollution*, 106: 268.

WADLEIGH, M.A., SCHWARCZ, H.P. and KRAMER, J.R. 1996. Isotopic evidence for the origin of sulphate in coastal rain. *Tellus, Series B*, 48: 44.

WHELPDALE, D.M. 1992. An overview of the atmospheric sulphur cycle. pp. 5-26 *In* HOWARTH, R.W., STEWART, J.W.B. and IVANOV, M.V. (Eds). Sulphur cycling on the continents: wetlands, terrestrial ecosystems and associated water bodies. SCOPE 48. John Wiley and Sons, Chichester. 350 pp.

WILSON, L.G., BRESSAN, R.A. and FILNER, P. 1978. Light-dependent emission of hydrogen sulfide from plants. *Plant Physiology*, 61: 184.

WINNER, W.E. 1988. Responses of bryophytes to air pollution. pp. 141-173 *In* NASH III, T.H. and WIRTH, V. (Eds). Lichens, bryophytes and air quality. Bibliotheca Lichenologica, 30. J. Cramer, Berlin-Stuttgart.

WINNER, W.E., BEWLEY, J.D., KROUSE, H.R. and BROWN, H.M. 1978. Stable sulfur isotope analysis of $SO_2$ pollution impact on vegetation. *Oecologia* (Berlin), 36: 351.

WINNER, W.E., SMITH, C.L., KOCH, G.W., MOONEY, H.A., BEWLEY, J.D. and KROUSE, H.R. 1971. Rates of emission of $H_2S$ from plants and patterns of stable sulphur isotope fractionation. *Nature*, 289: 672.

WIRTH, V. 1972. Die Silikatflechten-Gemeinschaften im ausseralpinen Zentraleuropa. *Dissertation Botanishche Lehre*, 17: 1-306.

WIRTH, V. and TÜRK, R. 1974. Über die $SO_2$-Resistenz von Flechten und die mit ihr interferienden Faktoren. Verhandlung der Gesellschaft für Ökologie.

WISEMAN, R.D. 1999. Monitoring changes in the sulphur isotopic composition and concentration of transplanted pendulous epiphytic lichens. M.Sc. Dissertation, Memorial University of Newfoundland. 164 pp.

WISEMAN, R.D. and WADLEIGH, M.A. 2002. Lichen response to changes in atmospheric sulphur: isotopic evidence. *Environmental Pollution*, 116 (2): 235.

WU, J. 1981. Evidence of sea spray produced by bursting bubbles. *Science*, 212: 324.

XUE, H.B., STUMM, W. and SIGG, L. 1988. The binding of heavy metals to algal surfaces. *Water Research*, 22: 917.

YUN, M. 2000. Direct measurement of sulphur isotope composition in lichens by continuous flow-isotope ratio mass spectrometry (CF-IRMS). M. Sc. Dissertation, Memorial University of Newfoundland. 165 pp.

ZAMBRANO, A. and NASH III, T.H. 2000. Lichen responses to short-term transplantation in Desierto de los Leones, Mexico City. *Environmental Pollution*, 107: 407.

ZAMBRANO, A., NASH III, T.H. and GRIES, C. 1999. Physiological effects of the Mexico City atmosphere on lichen transplants on oaks. *Journal of Environmental Quality*, 28 (5): 1548.

ZAKSHEK, E.M., PUCKETT, K.J. and PERCY, K.E. 1986. Lichen sulphur and lead levels in relation to deposition patterns in Eastern Canada. Water, Air and Soil pollution, 30: 161.

## Appendix I

### Calibration procedure for sulphur isotopic composition

The following table is an example of sulphur isotopic composition measurements for standard materials. The "raw" $\delta^{34}S$ is the isotopic composition measured by the instrument, whereas the $\delta^{34}S_{VCDT}$ is the known isotopic composition. The dashed line separates standards introduced at the beginning of the run from those introduced at the end.

| ID | Raw $\delta^{34}S$ (‰) | $\delta^{34}S_{VCDT}$ (‰) |
|---|---|---|
| IAEA-S-1 | - 2.01 | - 0.3 |
| IAEA-S-1 | - 2.67 | - 0.3 |
| IAEA-S-2 | +17.72 | +21.0 |
| IAEA-S-2 | +17.43 | +21.0 |
| IAEA-S-1 | - 1.69 | -0.3 |
| IAEA-S-1 | - 1.91 | -0.3 |
| IAEA-S-2 | +17.26 | +21.0 |
| IAEA-S-2 | +17.49 | +21.0 |

*Step 1. Determination of linear function equation*

Once the raw isotopic compositions for the standards are obtained, a linear function in the form of $y = mx + b$ can be calculated, where x is the measured isotopic composition and y is the calibrated isotopic composition.

From the previous data, this yields:

$$\delta^{34}S_{VCDT} = 1.0890165 \cdot \delta^{34}S_{raw} + 1.962939$$

*Step 2. Calculation of sample isotopic composition from the linear function.*

For a sample with a measured (raw) isotopic composition of 5.73 ‰, the calibrated isotopic composition is:

$$\delta^{34}S_{VCDT} = 1.0890165 \bullet 5.73 + 1.962939 = \mathbf{7.93\ ‰}$$

**Calibration procedure for sulphur concentration**

The area under the sulphur signal peak (in volts • second) obtained by the instrument is proportionally related to the total sulphur content. A linear regression curve can be obtained by introducing different-sized samples of an elemental reference material into the system, in this case BBOT, which has a sulphur content of 7.44% (w/w). The following table contains sample data for sulphur content calibration

| ID | Weight sample (mg) | [S] (% w/w) | Amt. S-cal (mg) | Area S (Vs) | Area S-cal (Vs) |
|---|---|---|---|---|---|
| BBOT | 0.396 | 7.44 | *0.0295* | 19.730 | *20.24* |
| BBOT | 0.321 | 7.44 | *0.0239* | 15.597 | *15.89* |
| BBOT | 0.472 | 7.44 | *0.0351* | 25.359 | *24.65* |
| BBOT | 0.533 | 7.44 | *0.0397* | 28.445 | *28.19* |
| BBOT | 0.709 | 7.44 | *0.0527* | 38.019 | *38.40* |
| BBOT | 0.913 | 7.44 | *0.0679* | 50.412 | *50.24* |
| BBOT | 0.538 | 7.44 | *0.0400* | 28.279 | *28.48* |
| BBOT | 0.639 | 7.44 | *0.475* | 34.227 | *34.34* |

*Step 1. Calculation of the amount of sulphur*

The amount of sulphur in a sample is a function of the sulphur concentration and the sample weight, according to the following equation:

Amount S (mg) = [S] (%) • sample weight (mg)

For a sample with a weight of 0.396 mg, the amount of sulphur is calculated as:

Amount S (mg) = 7.44 % * 0.396 mg = **0.0295 mg**

*Step 2. Determination of linear function equation*

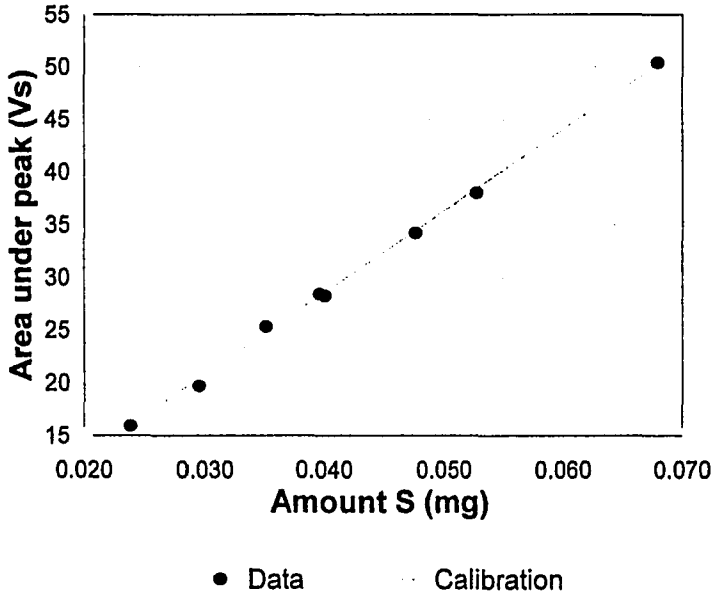
The relationship between the area under the sulphur peak and the amount of sulphur in the sample is directly proportional and can be represented with the equation $y = mx + b$, where x is the amount of sulphur and y is the area under the peak. Thus:

Area (Vs) = $m$ • amount S (mg) + $b$

For the previous data, the equation is:

Area (Vs) = 779.76432 • amount S (mg) - 2.731925

A graphical representation of the previous data yields:



*Step 3. Calculation of the sulphur content of a sample*

For a lichen sample with a mass of 14.983 mg and a measured area under the peak of 8.63 Vs, the amount of sulphur in the sample is calculated by:

(Area (Vs) + 2.731925) / 779.76432 = (8.63 + 2.731925) / 779.76432 = **0.014571 mg**

converted into units of parts per million (ppm), this yields:

$$= \frac{0.014571 \text{ mg S}}{14.983 \text{ mg}} \bullet \frac{1\,000\,000 \text{ mg}}{1 \text{ kg}} = \mathbf{973 \text{ ppm}}$$

# Appendix II

Table II.1: $\delta^{34}S_{VCDT}$ and [S] results.

| Month | MUN Botanical Garden | | | | Riverhead | | | |
|---|---|---|---|---|---|---|---|---|
| | ID | Weight (mg) | $\delta^{34}S_{VCDT}$ (‰) | [S] (ppm) | ID | Weight (mg) | $\delta^{34}S_{VCDT}$ (‰) | [S] (ppm) |
| 0 | 0-BG-C1 | 367.4 | 5.1 | 845 | 0-RH-C1 | 1250.9 | 15.8 | 418 |
| | 0-BG-C2 | 421.6 | 7.8 | 535 | 0-RH-C2 | 1558.8 | 16.8 | 452 |
| | 0-BG-C3 | 807.2 | 7.6 | 659 | 0-RH-C3 | 904.9 | 17.9 | 489 |
| | 0-BG-C4 | 275.2 | 7.4 | 743 | 0-RH-C4 | 801.5 | 16.4 | 460 |
| | 0-BG-C5 | 241.8 | 8.5 | 525 | 0-RH-C5 | 664.8 | 18.3 | 432 |
| | 0-BG-C6 | 558.3 | 7.9 | 772 | 0-RH-C6 | 1438.7 | 15.5 | 417 |
| | 0-BG-C7 | 546.6 | 6.8 | 795 | 0-RH-C7 | 1028.2 | 17.8 | 404 |
| | 0-BG-C8 | 540.3 | 6.9 | 788 | 0-RH-C8 | 970 | 17.7 | 432 |
| 1 | 1-BG-T6 | 268 | 14.4 | 629 | 1-RH-T19 | 458.7 | 7.8 | 869 |
| | 1-BG-T15 | 204.7 | 13.3 | 678 | 1-RH-T25 | 2442.1 | 9.9 | 675 |
| | 1-BG-T16 | 145.7 | 11.5 | 646 | 1-RH-T26 | 585.7 | 8.7 | 585 |
| | 1-BG-T21 | 1400.7 | 14.4 | 565 | 1-RH-T7 | 279.4 | 6.2 | 665 |
| | 1-BG-T11 | 522.3 | 13.8 | 673 | 1-RH-T15 | 896.8 | 9.6 | 534 |
| | 1-BG-TC15 | 622.1 | 7.0 | 688 | 1-RH-TC13 | 361.4 | 16.9 | 487 |
| | 1-BG-TC20 | 348.7 | 5.1 | 674 | 1-RH-TC20 | 387 | 16.6 | 527 |
| | 1-BG-C1 | 429.7 | 4.9 | 839 | 1-RH-C1 | 760.7 | 17.8 | 600 |
| | 1-BG-C2 | 340.7 | 6.0 | 624 | 1-RH-C2 | 1244.6 | 18.3 | 470 |
| | 1-BG-C3 | 356.4 | 5.6 | 717 | 1-RH-C3 | 456.5 | 8.9 | 648 |
| 2 | 2-BG-T14 | 214 | 12.2 | 653 | 2-RH-T24 | 236.1 | 10.5 | 731 |
| | 2-BG-T9 | 865.1 | 12.7 | 457 | 2-RH-T20 | 357.3 | 8.9 | 509 |
| | 2-BG-T15 | 377.4 | 10.1 | 544 | 2-RH-T22 | 417.3 | 8.6 | 629 |
| | 2-BG-T13 | 168.7 | 10.7 | 535 | 2-RH-T11 | 943.1 | 7.9 | 558 |
| | 2-BG-TC22 | 189.8 | 5.5 | 879 | 2-RH-T4 | 322.5 | 8.9 | 706 |
| | 2-BG-TC7 | 223.4 | 5.3 | 811 | 2-RH-TC7 | 380.6 | 16.3 | 443 |
| | 2-BG-TC20 | 505.9 | 6.6 | 637 | 2-RH-TC5 | 273.2 | 17.0 | 531 |
| | 2-BG-C1 | 205.6 | 5.9 | 611 | 2-RH-C1 | 1315.7 | 16.9 | 447 |
| | 2-BG-C2 | 436.9 | 6.8 | 579 | 2-RH-C2 | 187 | 16.6 | 493 |
| | 2-BG-C3 | 875.5 | 5.6 | 615 | 2-RH-C3 | 1234.6 | 18.2 | 411 |
| 3 | 3-BG-T23 | 259.9 | 15.2 | 520 | 3-RH-T30 | 590.7 | 9.7 | 589 |
| | 3-BG-T22 | 108.2 | 9.2 | 672 | 3-RH-T19 | 349 | 8.4 | 670 |

Table II.1 (continued)

| Month | MUN Botanical Garden | | | | Riverhead | | | |
|---|---|---|---|---|---|---|---|---|
| | ID | Weight (mg) | $\delta^{34}S_{VCDT}$ (‰) | [S] (ppm) | ID | Weight (mg) | $\delta^{34}S_{VCDT}$ (‰) | [S] (ppm) |
| | 3-BG-T28 | 172.8 | 11.1 | 661 | 3-RH-T2 | 123.6 | 9.7 | 738 |
| | 3-BG-TC17 | 253.3 | 6.9 | 988 | 3-RH-T14 | 158.5 | 17.3 | 522 |
| | 3-BG-TC16 | 363.1 | 6.4 | 825 | 3-RH-TC18 | 507.9 | 14.5 | 425 |
| | 3-BG-TC13 | 152.9 | 5.3 | 942 | 3-RH-TC19 | 313.6 | 17.6 | 596 |
| | 3-BG-TC9 | 784.2 | 6.4 | 611 | 3-RH-TC3 | 952.7 | 16.4 | 621 |
| | 3-BG-C1 | 562.6 | 5.5 | 887 | 3-RH-C1 | 400 | 17.3 | 520 |
| | 3-BG-C2 | 395.4 | 7.7 | 582 | 3-RH-C2 | 312.4 | 17.0 | 616 |
| | 3-BG-C3 | 483.5 | 7.2 | 770 | 3-RH-C3 | 289.7 | 17.0 | 659 |
| 4 | 4-BG-T15 | 331.5 | 14.5 | 498 | 4-RH-T5 | 178.7 | 10.2 | 582 |
| | 4-BG-T17 | 498.9 | 14.5 | 516 | 4-RH-T10 | 211.8 | 19.0 | 539 |
| | 4-BG-T20 | 167.2 | 13.2 | 478 | 4-RH-T17 | 525.1 | 8.7 | 595 |
| | 4-BG-T21 | 330.4 | 11.8 | 528 | 4-RH-T24 | 441.3 | 8.7 | 630 |
| | 4-BG-T22 | 394.4 | 10.3 | 560 | 4-RH-T30 | 133 | 9.0 | 638 |
| | 4-BG-T25 | 824.4 | 13.0 | 458 | 4-RH-TC4 | 251.8 | 18.9 | 347 |
| | 4-BG-TC17 | 242.7 | 6.5 | 713 | 4-RH-TC28 | 743.5 | 16.2 | 405 |
| | 4-BG-C1 | 231.6 | 6.5 | 648 | 4-RH-C1 | 132.1 | 16.3 | 542 |
| | 4-BG-C2 | 160.6 | 6.4 | 662 | 4-RH-C2 | 90.5 | 16.0 | 405 |
| | 4-BG-C3 | 456.5 | 6.1 | 621 | 4-RH-C3 | 270.4 | 16.7 | 566 |
| 5 | 5-BG-T10 | 633.4 | 14.1 | 690 | 5-RH-T1 | 357.2 | 7.6 | 734 |
| | 5-BG-T12 | 112.8 | 6.7 | 731 | 5-RH-T6 | 194.8 | 8.4 | 575 |
| | 5-BG-T18 | 201.1 | 13.5 | 657 | 5-RH-T9 | 563.4 | 12.4 | 577 |
| | 5-BG-T29 | 526.3 | 10.3 | 626 | 5-RH-T10 | 833.5 | 9.7 | 757 |
| | 5-BG-TC8 | 556.1 | 6.6 | 816 | 5-RH-T18 | 800 | 9.0 | 624 |
| | 5-BG-TC9 | 444.1 | 6.4 | 851 | 5-RH-T27 | 150.1 | 8.3 | 568 |
| | 5-BG-TC20 | 230.4 | 6.6 | 851 | 5-RH-TC4 | 115.4 | 16.4 | 460 |
| | 5-BG-C1 | 218.3 | 6.8 | 812 | 5-RH-C1 | 248.2 | 14.9 | 475 |
| | 5-BG-C2 | 339 | 7.2 | 807 | 5-RH-C2 | 272.5 | 17.7 | 514 |
| | 5-BG-C3 | 644.8 | 7.1 | 826 | 5-RH-C3 | 299.3 | 14.7 | 521 |
| 6 | 6-BG-T12 | 147.7 | 12.7 | 536 | 6-RH-T3 | 451.6 | 10.4 | 549 |
| | 6-BG-T16 | 155.4 | 10.8 | 518 | 6-RH-T10 | 369.6 | 18.3 | 557 |
| | 6-BG-T18 | 465.7 | 12.7 | 672 | 6-RH-T18 | 157.4 | 10.9 | 550 |
| | 6-BG-T19 | 114.3 | 10.9 | 372 | 6-RH-T27 | 432.1 | 9.7 | 783 |

Table II.1 (continued)

| Month | MUN Botanical Garden | | | | Riverhead | | | |
|---|---|---|---|---|---|---|---|---|
| | ID | Weight (mg) | $\delta^{34}S_{VCDT}$ (‰) | [S] (ppm) | ID | Weight (mg) | $\delta^{34}S_{VCDT}$ (‰) | [S] (ppm) |
| | 6-BG-T25 | 255.9 | 13.0 | 431 | 6-RH-TC1 | 394.3 | 17.5 | 345 |
| | 6-BG-T29 | 179.9 | 11.4 | 448 | 6-RH-TC8 | 226 | 18.3 | 392 |
| | 6-BG-TC14 | 148.8 | 5.1 | 732 | 6-RH-TC20 | 472.4 | 16.6 | 397 |
| | 6-BG-C1 | 101.7 | 5.1 | 645 | 6-RH-C1 | 309.8 | 19.2 | 423 |
| | 6-BG-C2 | 196.6 | 7 | 711 | 6-RH-C2 | 400.7 | 17.5 | 418 |
| | 6-BG-C3 | 193.3 | 6.4 | 763 | 6-RH-C3 | 348.8 | 15.9 | 364 |
| 7 | 7-BG-T2 | 366.9 | 10.1 | 583 | 7-RH-T3 | 1037.8 | 8.9 | 525 |
| | 7-BG-T4 | 757.6 | 11.8 | 504 | 7-RH-T5 | 140.4 | 8.5 | 667 |
| | 7-BG-T14 | 589.2 | 10.0 | 547 | 7-RH-T12 | 859.8 | 9.0 | 498 |
| | 7-BG-TC8 | 999.3 | 6.6 | 644 | 7-RH-T25 | 995.5 | 9.6 | 545 |
| | 7-BG-TC8 | 674.1 | 6.4 | 531 | 7-RH-TC8 | 430.1 | 17.6 | 422 |
| | 7-BG-TC15 | 284.8 | 6.6 | 788 | 7-RH-TC16 | 710 | 15.8 | 449 |
| | 7-BG-TC22 | 511.3 | 5.4 | 837 | 7-RH-TC28 | 596 | 16.7 | 473 |
| | 7-BG-C1 | 484 | 6.8 | 636 | 7-RH-C1 | 431.7 | 15.0 | 417 |
| | 7-BG-C2 | 330.2 | 4.7 | 769 | 7-RH-C2 | 744.9 | 17.6 | 445 |
| | 7-BG-C3 | 452.5 | 4.8 | 907 | 7-RH-C3 | 448.7 | 15.5 | 473 |
| 8 | 8-BG-T14 | 454.7 | 12.5 | 566 | 8-RH-T3 | 498.6 | 9.9 | 558 |
| | 8-BG-T19 | 200.6 | 11.9 | 495 | 8-RH-T4 | 706.1 | 9.6 | 465 |
| | 8-BG-T20 | 316.7 | 11.5 | 564 | 8-RH-T5 | 519.6 | 9.8 | 521 |
| | 8-BG-T23 | 2151.3 | 13.4 | 568 | 8-RH-T13 | 631.7 | 9.7 | 574 |
| | 8-BG-T26 | 936.3 | 12.6 | 407 | 8-RH-T19 | 408.5 | 9.4 | 590 |
| | 8-BG-T28 | 371.5 | 11.5 | 525 | 8-RH-T29 | 532.8 | 9.2 | 641 |
| | 8-BG-TC9 | 436.7 | 6.8 | 599 | 8-RH-TC5 | 358.1 | 16.1 | 514 |
| | 8-BG-C1 | 348.9 | 6.8 | 934 | 8-RH-C1 | 642.8 | 17.2 | 438 |
| | 8-BG-C2 | 675.2 | 6.5 | 726 | 8-RH-C2 | 516.1 | 14.9 | 490 |
| | 8-BG-C3 | 458.2 | 6.2 | 664 | 8-RH-C3 | 237.2 | 14.7 | 491 |
| 9 | 9-BG-T1 | 328.9 | 12.3 | 470 | 9-RH-T8 | 274.7 | 9.6 | 698 |
| | 9-BG-T4 | 464.2 | 11.6 | 601 | 9-RH-T12 | 437.5 | 11.3 | 608 |
| | 9-BG-T8 | 315.6 | 12.2 | 530 | 9-RH-T21 | 373.8 | 10.1 | 636 |
| | 9-BG-T16 | 356.8 | 8.0 | 652 | 9-RH-T26 | 893.5 | 9.1 | 544 |
| | 9-BG-T24 | 665 | 11.1 | 559 | 9-RH-TC13 | 807.1 | 17.0 | 573 |
| | 9-BG-TC13 | 202.9 | 3.4 | 874 | 9-RH-TC16 | 552.9 | 16.1 | 501 |

Table II.1: (continued)

| Month | MUN Botanical Garden | | | | Riverhead | | | |
|---|---|---|---|---|---|---|---|---|
| | ID | Weight (mg) | $\delta^{34}S_{VCDT}$ (‰) | [S] (ppm) | ID | Weight (mg) | $\delta^{34}S_{VCDT}$ (‰) | [S] (ppm) |
| | 9-BG-TC14 | 297.8 | 7.3 | 708 | 9-RH-TC19 | 389.1 | 16.3 | 476 |
| | 9-BG-C1 | 776.3 | 9.2 | 480 | 9-RH-C1 | 658.3 | 16.0 | 495 |
| | 9-BG-C2 | 322.4 | 5.8 | 647 | 9-RH-C2 | 280.6 | 16.9 | 566 |
| | 9-BG-C3 | 481.1 | 7.9 | 604 | 9-RH-C3 | 183.3 | 16.2 | 513 |
| 10 | 10-BG-T3 | 733.9 | 11.6 | 518 | 10-RH-T6 | 426.6 | 11.3 | 643 |
| | 10-BG-T12 | 449.5 | 11.6 | 591 | 10-RH-T9 | 540.8 | 10.6 | 558 |
| | 10-BG-T17 | 251.2 | 12.5 | 632 | 10-RH-T16 | 425.2 | 10.3 | 732 |
| | 10-BG-T20 | 471.4 | 13.1 | 560 | 10-RH-T17 | 383.7 | 9.8 | 710 |
| | 10-BG-T23 | 731.5 | 14.1 | 624 | 10-RH-T23 | 493.4 | 10.4 | 597 |
| | 10-BG-T26 | 548.7 | 11.3 | 547 | 10-RH-TC1 | 224.4 | 16.0 | 619 |
| | 10-BG-TC13 | 295.7 | 5.5 | 874 | 10-RH-TC7 | 638.2 | 15.9 | 643 |
| | 10-BG-C1 | 556.5 | 6.6 | 641 | 10-RH-C1 | 195.4 | 16.3 | 525 |
| | 10-BG-C2 | 261.6 | 5.3 | 810 | 10-RH-C2 | 245.4 | 15.6 | 629 |
| | 10-BG-C3 | 275.8 | 6.7 | 798 | 10-RH-C3 | 427.1 | 18.2 | 636 |
| 11 | 11-BG-T4 | 441.4 | 12.0 | 638 | 11-RH-T1 | 293.9 | 13.5 | 622 |
| | 11-BG-T5 | 740 | 12.2 | 583 | 11-RH-T8 | 340.8 | 11.9 | 577 |
| | 11-BG-T9 | 818.9 | 10.7 | 722 | 11-RH-T11 | 394.7 | 12.8 | 626 |
| | 11-BG-T24 | 127.4 | 13.7 | 596 | 11-RH-T15 | 192 | 13.5 | 395 |
| | 11-BG-T28 | 239.6 | 14.7 | 606 | 11-RH-T16 | 602.3 | 11.2 | 573 |
| | 11-BG-T30 | 625.2 | 10.9 | 755 | 11-RH-T22 | 327.8 | 12.5 | 455 |
| | 11-BG-TC15 | 207.1 | 7.6 | 785 | 11-RH-TC20 | 534.2 | 18.7 | 546 |
| | 11-BG-C1 | 490.3 | 5.2 | 756 | 11-RH-C1 | 505.4 | 18.7 | 389 |
| | 11-BG-C2 | 644.3 | 4.1 | 949 | 11-RH-C2 | 179.2 | 18.8 | 545 |
| | 11-BG-C3 | 169 | 5.4 | 1007 | 11-RH-C3 | 206 | 17.4 | 499 |
| 12 | 12-BG-T11 | 254.5 | 10.9 | 761 | 12-RH-T2 | 290.4 | 11.8 | 474 |
| | 12-BG-T13 | 293.4 | 11.0 | 475 | 12-RH-T8 | 286.6 | 12.7 | 552 |
| | 12-BG-T17 | 366 | 11.3 | 385 | 12-RH-T20 | 568.3 | 12.8 | 472 |
| | 12-BG-T30 | 612.9 | 11.8 | 476 | 12-RH-T28 | 290.6 | 13.6 | 521 |
| | 12-BG-TC7 | 616.3 | 6.4 | 697 | 12-RH-TC3 | 289.5 | 17.8 | 640 |
| | 12-BG-TC16 | 314.4 | 7.5 | 798 | 12-RH-TC16 | 1272 | 16.3 | 518 |
| | 12-BG-TC26 | 620.9 | 6.7 | 678 | 12-RH-TC19 | 470.9 | 20.0 | 371 |
| | 12-BG-C1 | 203.8 | 6.6 | 722 | 12-RH-C1 | 191.5 | 16.8 | 590 |

Table II.1: (continued)

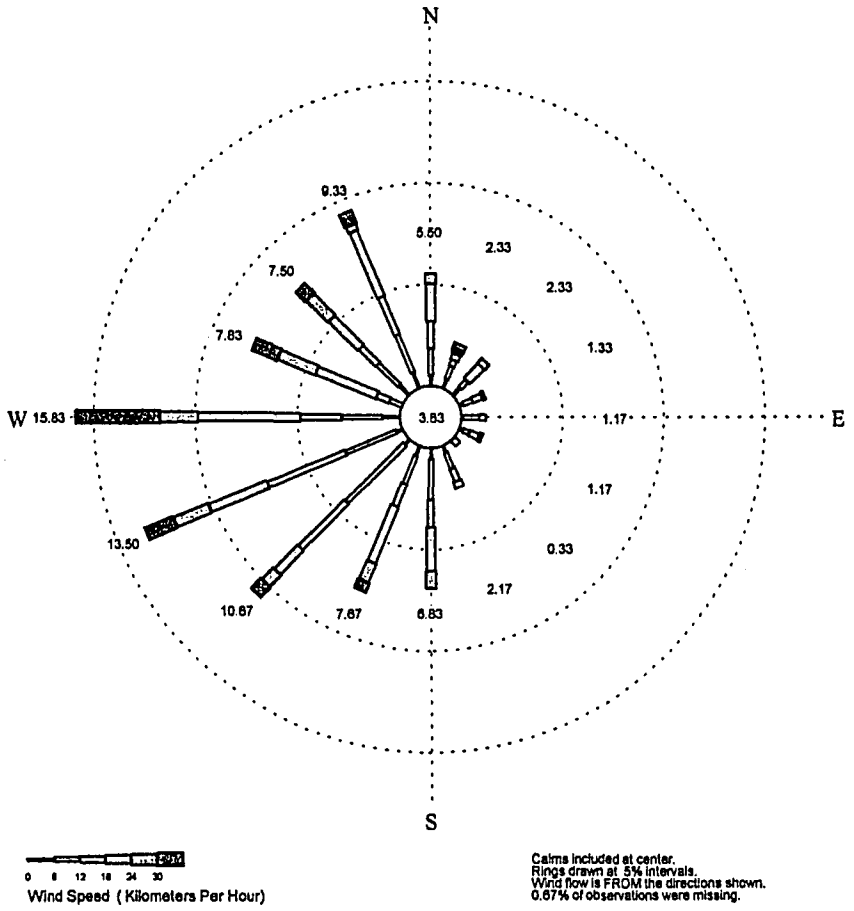
| Month | MUN Botanical Garden | | | | Riverhead | | | |
|---|---|---|---|---|---|---|---|---|
| | ID | Weight (mg) | $\delta^{34}S_{VCDT}$ (‰) | [S] (ppm) | ID | Weight (mg) | $\delta^{34}S_{VCDT}$ (‰) | [S] (ppm) |
| | 12-BG-C2 | 354.1 | 6.5 | 658 | 12-RH-C2 | 375.5 | 16.8 | 405 |
| | 12-BG-C3 | 322.8 | 7.6 | 650 | 12-RH-C3 | 185 | 17.5 | 632 |
| 13 | 13-BG-T2 | 408.3 | 12.3 | 477 | 13-RH-T9 | 247.4 | 12.9 | 399 |
| | 13-BG-T4 | 374.8 | 11.5 | 516 | 13-RH-T17 | 235.7 | 10.8 | 549 |
| | 13-BG-T7 | 1110.9 | 12.0 | 457 | 13-RH-T29 | 242 | 12.8 | 675 |
| | 13-BG-T26[a] | NA | NA | NA | 13-RH-T30 | 310.7 | 11.5 | 374 |
| | 13-BG-T29 | 277.6 | 13.2 | 541 | 13-RH-TC1 | 527.5 | 17.5 | 342 |
| | 13-BG-TC16 | 360.9 | 7.2 | 792 | 13-RH-TC3 | 145.1 | 14.8 | 545 |
| | 13-BG-TC17 | 375 | 6.2 | 926 | 13-RH-TC28 | 563.8 | 16.8 | 387 |
| | 13-BG-C1 | 582.9 | 6.7 | 483 | 13-RH-C1 | 380.7 | 15.9 | 495 |
| | 13-BG-C2 | 355.7 | 6.0 | 895 | 13-RH-C2 | 1098.7 | 19.3 | 407 |
| | 13-BG-C3 | 171.1 | 5.9 | 995 | 13-RH-C3 | 938.5 | 18.0 | 478 |
| 14 | 14-BG-T1 | 667.6 | 10.4 | 500 | 14-RH-T1 | 817.5 | 13.1 | 515 |
| | 14-BG-T18 | 222.6 | 9.4 | 467 | 14-RH-T2 | 328.8 | 12.8 | 530 |
| | 14-BG-T19 | 122.1 | 9.6 | 691 | 14-RH-T6 | 1161.2 | 12.6 | 610 |
| | 14-BG-T21 | 334.4 | 10.0 | 661 | 14-RH-T11 | 297.5 | 11.8 | 669 |
| | 14-BG-T24 | 161.6 | 10.2 | 621 | 14-RH-T15 | 255.8 | 14.0 | 638 |
| | 14-BG-T27 | 101.1 | 11.4 | 659 | 14-RH-T22 | 195.3 | 13.2 | 607 |
| | 14-BG-TC7 | 647.2 | 5.8 | 990 | 14-RH-TC7 | 2211.9 | 16.3 | 505 |
| | 14-BG-C1 | 431.3 | 6.9 | 756 | 14-RH-C1 | 408 | 16.1 | 527 |
| | 14-BG-C2 | 213 | 6.0 | 914 | 14-RH-C2 | 402.5 | 17.4 | 639 |
| | 14-BG-C3 | 110.8 | 5.8 | 1170 | 14-RH-C3 | 462.6 | 17.7 | 632 |
| 15 | 15-BG-T2 | 2026.5 | 10.8 | 671 | 15-RH-T7 | 644 | 12.5 | 711 |
| | 15-BG-T5 | 421.5 | 9.1 | 761 | 15-RH-T12 | 161.9 | 12.8 | 693 |
| | 15-BG-T6 | 2474.2 | 11.8 | 716 | 15-RH-T14 | 296.7 | 12.5 | 644 |
| | 15-BG-T8 | 521.5 | 11.6 | 821 | 15-RH-T16 | 323.5 | 10.7 | 665 |
| | 15-BG-T9 | 298.2 | 9.6 | 857 | 15-RH-T23 | 730.5 | 11.2 | 620 |
| | 15-BG-TC5 | 400.6 | 7.4 | 829 | 15-RH-T26 | 339.5 | 10.3 | 574 |
| | 15-BG-TC26 | 972.5 | 11.6 | 644 | 15-RH-TC8 | 164.2 | 16.2 | 625 |
| | 15-BG-C1 | 752.4 | 6.3 | 944 | 15-RH-C1 | 185.5 | 17.4 | 815 |
| | 15-BG-C2 | 241.3 | 5.8 | 1004 | 15-RH-C2 | 507.6 | 16.0 | 642 |

Table II.1: (continued)

| Month | MUN Botanical Garden | | | | Riverhead | | | |
|---|---|---|---|---|---|---|---|---|
| | ID | Weight (mg) | $\delta^{34}S_{VCDT}$ (‰) | [S] (ppm) | ID | Weight (mg) | $\delta^{34}S_{VCDT}$ (‰) | [S] (ppm) |
| | 15-BG-C3 | 308.2 | 5.2 | 1074 | 15-RH-C3 | 663.8 | 16.0 | 662 |
| 16 | 16-BG-T1 | 289 | 9.8 | 802 | 16-BG-T7 | 285.5 | 12.2 | 760 |
| | 16-BG-T7 | 606.5 | 9.5 | 674 | 16-BG-T13 | 486.2 | 10.6 | 710 |
| | 16-BG-T8 | 502.7 | 9.9 | 878 | 16-RH-T20 | 390 | 12.8 | 674 |
| | 16-BG-T13 | 325.3 | 8.8 | 678 | 16-RH-T21 | 504.1 | 12.5 | 646 |
| | 16-BG-T22 | 564.4 | 9.3 | 709 | 16-RH-T29 | 918.7 | 10.6 | 732 |
| | 16-BG-T27 | 406.2 | 9.8 | 730 | 16-RH-TC4 | 661.5 | 16.5 | 623 |
| | 16-BG-TC22 | 480.1 | 6.3 | 957 | 16-RH-TC5 | 229.9 | 16.0 | 715 |
| | 16-BG-C1 | 274.7 | 5.4 | 940 | 16-RH-C1 | 147.4 | 18.3 | 754 |
| | 16-BG-C2 | 285.1 | 6.7 | 1028 | 16-RH-C2 | 292.5 | 16.1 | 777 |
| | 16-BG-C3 | 586.5 | 6.0 | 1040 | 16-RH-C3 | 684.3 | 16.6 | 706 |
| 17 | 17-BG-T3 | 535.8 | 9.7 | 673 | 17-RH-T13 | 1346.3 | 12.3 | 428 |
| | 17-BG-T5 | 237.7 | 8.9 | 632 | 17-RH-T18 | 192.1 | 17 | 466 |
| | 17-BG-T10 | 233.6 | 9.5 | 728 | 17-RH-T23 | 314.7 | 12.0 | 662 |
| | 17-BG-T25 | 373.3 | 9.6 | 644 | 17-RH-T25 | 419.3 | 11.7 | 751 |
| | 17-BG-T27 | 142.2 | 8.7 | 674 | 17-RH-T28 | 313.6 | 13.6 | 524 |
| | 17-BG-TC5 | 260.1 | 5.7 | 854 | 17-RH-TC13 | 288.3 | 17.4 | 485 |
| | 17-BG-TC26 | 470.4 | 5.8 | 853 | 17-RH-TC18 | 715.9 | 15.4 | 456 |
| | 17-BG-C1 | 216.8 | 6.2 | 465 | 17-RH-C1 | 340.8 | 16.7 | 430 |
| | 17-BG-C2 | 395 | 5.2 | 641 | 17-RH-C2 | 281.4 | 15.4 | 576 |
| | 17-BG-C3 | 488 | 5.4 | 855 | 17-RH-C3 | 444.9 | 14.5 | 521 |
| 18 | 18-BG-T3 | 421 | 9.0 | 624 | 18-RH-T4 | 192.4 | 11.4 | 600 |
| | 18-BG-T6 | 720.8 | 10.7 | 614 | 18-RH-T14 | 327.8 | 12.8 | 417 |
| | 18-BG-T10 | 503.3 | 7.9 | 810 | 18-RH-T21 | 12.7 | 12.7 | 507 |
| | 18-BG-T11 | 414 | 7.3 | 710 | 18-RH-T24 | 246 | 11.7 | 626 |
| | 18-BG-T30 | 1037.1 | 10.0 | 613 | 18-RH-T27 | 763.4 | 16.4 | 356 |
| | 18-BG-TC5 | 533.5 | 6.0 | 941 | 18-RH-T28 | 266.9 | 12.2 | 595 |
| | 18-BG-TC14 | 607.5 | 5.2 | 646 | 18-RH-TC18 | 259.5 | 16.9 | 491 |
| | 18-BG-C1 | 1231.4 | 4.6 | 1129 | 18-RH-C1 | 230.5 | 17.3 | 417 |
| | 18-BG-C2 | 189.2 | 3.8 | 805 | 18-RH-C2 | 205.1 | 17.3 | 465 |
| | 18-BG-C3 | 412.6 | 5.6 | 982 | 18-RH-C3 | 432.5 | 16.2 | 498 |

a: missing sample.

# Appendix III




PERCENT OCCURRENCE: Wind Speed ( Kilometers Per Hour)
LOWER BOUND OF CATEGORY

| DIR | 0 | 6 | 12 | 18 | 24 | 30 |
|---|---|---|---|---|---|---|
| N | 0.50 | 1.33 | 1.33 | 1.83 | 0.50 | 0.00 |
| NNE | 0.50 | 0.67 | 0.33 | 0.33 | 0.17 | 0.33 |
| NE | 0.50 | 0.50 | 0.00 | 1.00 | 0.33 | 0.00 |
| ENE | 0.17 | 0.50 | 0.50 | 0.17 | 0.00 | 0.00 |
| E | 0.00 | 0.83 | 0.33 | 0.00 | 0.00 | 0.00 |
| ESE | 0.17 | 0.33 | 0.50 | 0.17 | 0.00 | 0.00 |
| SE | 0.00 | 0.00 | 0.33 | 0.00 | 0.00 | 0.00 |
| SSE | 0.33 | 0.83 | 0.67 | 0.33 | 0.00 | 0.00 |

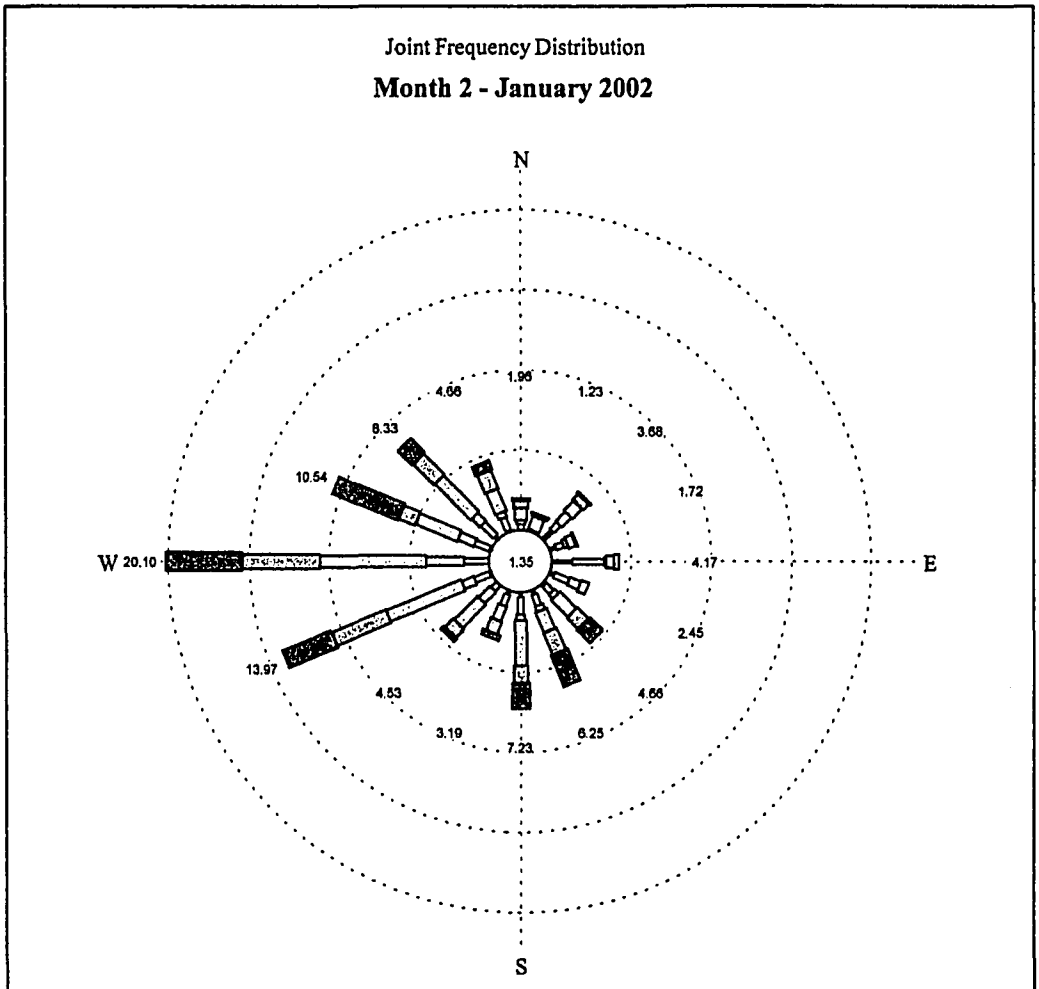
TOTAL OBS = 600 MISSING OBS = 4

PERCENT OCCURRENCE: Wind Speed ( Kilometers Per Hour)
LOWER BOUND OF CATEGORY

| DIR | 0 | 6 | 12 | 18 | 24 | 30 |
|---|---|---|---|---|---|---|
| S | 0.50 | 2.00 | 1.33 | 2.17 | 0.83 | 0.00 |
| SSW | 0.50 | 1.50 | 1.17 | 3.00 | 1.00 | 0.50 |
| SW | 0.33 | 4.00 | 3.17 | 1.67 | 0.83 | 0.67 |
| WSW | 0.33 | 2.67 | 4.17 | 3.00 | 1.83 | 1.50 |
| W | 0.83 | 2.00 | 2.00 | 5.00 | 1.83 | 4.17 |
| WNW | 0.00 | 0.67 | 0.67 | 3.17 | 2.00 | 1.33 |
| NW | 0.67 | 1.50 | 1.17 | 2.00 | 1.50 | 0.67 |
| NNW | 0.83 | 2.00 | 2.00 | 3.50 | 0.33 | 0.67 |

CALM OBS = 23 PERCENT CALM = 3.83

Figure III.1: Wind rose diagram for St. John's, Month 1 (13.11.2001 - 07.12.2001).




PERCENT OCCURRENCE: Wind Speed ( Kilometers Per Hour)
LOWER BOUND OF CATEGORY

| DIR | 0 | 6 | 12 | 18 | 24 | 30 |
|---|---|---|---|---|---|---|
| N | 0.00 | 0.37 | 0.25 | 0.74 | 0.49 | 0.12 |
| NNE | 0.00 | 0.00 | 0.12 | 0.86 | 0.12 | 0.12 |
| NE | 0.74 | 0.61 | 0.86 | 0.86 | 0.49 | 0.12 |
| ENE | 0.37 | 0.49 | 0.25 | 0.49 | 0.12 | 0.00 |
| E | 1.23 | 1.96 | 0.12 | 0.49 | 0.37 | 0.00 |
| ESE | 0.25 | 0.98 | 0.74 | 0.49 | 0.00 | 0.00 |
| SE | 0.12 | 0.61 | 0.37 | 1.47 | 0.98 | 1.10 |
| SSE | 0.25 | 0.74 | 0.37 | 1.35 | 1.47 | 2.08 |

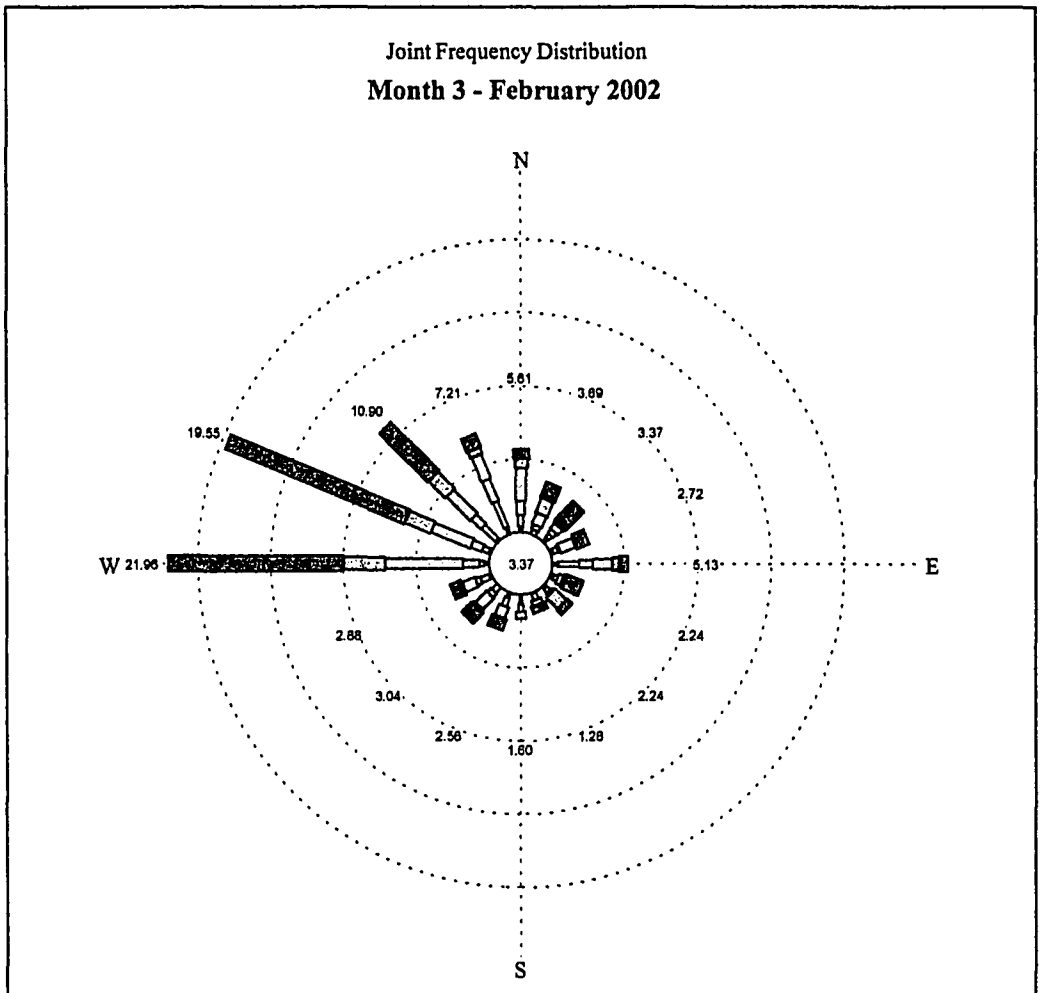
TOTAL OBS = 816 MISSING OBS = 0

PERCENT OCCURRENCE: Wind Speed ( Kilometers Per Hour)
LOWER BOUND OF CATEGORY

| DIR | 0 | 6 | 12 | 18 | 24 | 30 |
|---|---|---|---|---|---|---|
| S | 0.25 | 1.10 | 0.37 | 2.82 | 1.10 | 1.59 |
| SSW | 0.25 | 0.74 | 0.98 | 0.86 | 0.00 | 0.37 |
| SW | 0.00 | 0.49 | 0.98 | 2.08 | 0.74 | 0.25 |
| WSW | 0.12 | 0.98 | 0.86 | 5.02 | 3.68 | 3.31 |
| W | 0.00 | 1.59 | 2.33 | 6.62 | 4.90 | 4.66 |
| WNW | 0.12 | 0.98 | 1.10 | 2.82 | 1.10 | 4.41 |
| NW | 0.25 | 1.10 | 0.74 | 3.19 | 1.84 | 1.23 |
| NNW | 0.12 | 0.86 | 0.37 | 1.47 | 1.23 | 0.61 |

CALM OBS = 11 PERCENT CALM = 1.35

Figure III.2: Wind rose diagram for St. John's, Month 2 (10.12.2001 - 12.01.2001).




PERCENT OCCURRENCE: Wind Speed ( Kilometers Per Hour)
LOWER BOUND OF CATEGORY

| DIR | 0 | 6 | 12 | 18 | 24 | 30 |
|---|---|---|---|---|---|---|
| N | 0.32 | 0.80 | 0.96 | 2.24 | 0.64 | 0.64 |
| NNE | 0.00 | 0.32 | 0.32 | 0.80 | 1.12 | 1.12 |
| NE | 0.32 | 0.32 | 0.32 | 0.48 | 0.16 | 1.76 |
| ENE | 0.16 | 0.32 | 0.16 | 1.12 | 0.00 | 0.96 |
| E | 0.32 | 1.44 | 0.96 | 1.28 | 0.48 | 0.64 |
| ESE | 0.16 | 0.32 | 0.00 | 0.32 | 0.16 | 1.28 |
| SE | 0.16 | 0.32 | 0.16 | 0.00 | 1.12 | 0.48 |
| SSE | 0.00 | 0.48 | 0.00 | 0.32 | 0.00 | 0.48 |

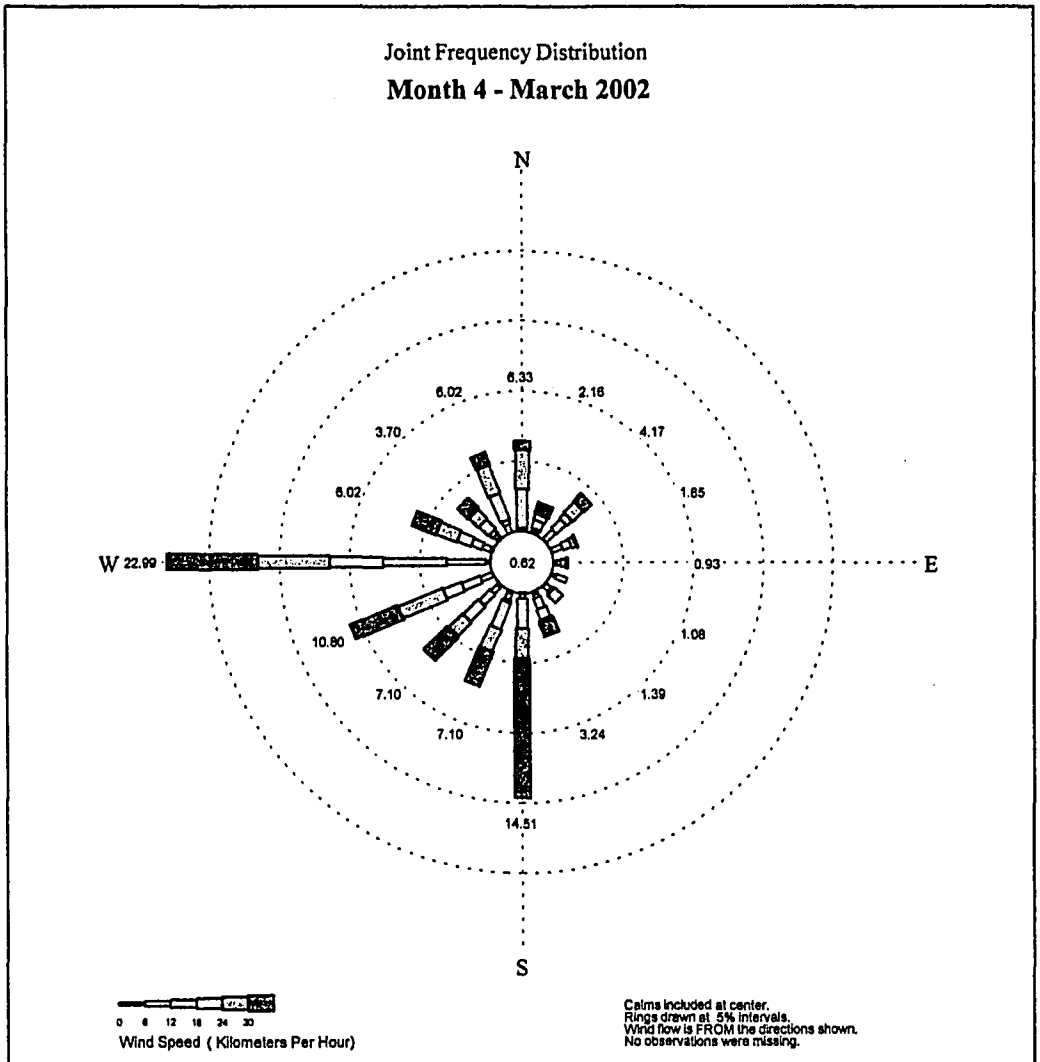
TOTAL OBS = 624 MISSING OBS = 4

PERCENT OCCURRENCE: Wind Speed ( Kilometers Per Hour)
LOWER BOUND OF CATEGORY

| DIR | 0 | 6 | 12 | 18 | 24 | 30 |
|---|---|---|---|---|---|---|
| S | 0.64 | 0.48 | 0.00 | 0.48 | 0.00 | 0.00 |
| SSW | 0.32 | 0.64 | 0.00 | 0.80 | 0.00 | 0.80 |
| SW | 0.00 | 0.48 | 0.32 | 1.12 | 0.16 | 0.96 |
| WSW | 0.16 | 0.64 | 0.32 | 0.96 | 0.00 | 0.80 |
| W | 0.32 | 0.32 | 1.12 | 5.29 | 2.88 | 12.02 |
| WNW | 0.16 | 0.48 | 0.80 | 2.88 | 1.92 | 13.30 |
| NW | 0.16 | 1.28 | 0.96 | 2.24 | 1.60 | 4.65 |
| NNW | 0.48 | 1.92 | 1.60 | 1.76 | 0.48 | 0.96 |

CALM OBS = 21 PERCENT CALM = 3.37

Figure III.3: Wind rose diagram for St. John's, Month 3 (14.01.2002 - 08.02.2002).




PERCENT OCCURRENCE: Wind Speed ( Kilometers Per Hour)
LOWER BOUND OF CATEGORY

| DIR | 0 | 6 | 12 | 18 | 24 | 30 |
|---|---|---|---|---|---|---|
| N | 0.00 | 0.00 | 0.15 | 2.78 | 2.78 | 0.62 |
| NNE | 0.00 | 0.15 | 0.15 | 0.62 | 0.46 | 0.77 |
| NE | 0.00 | 0.93 | 0.93 | 0.46 | 1.08 | 0.77 |
| ENE | 0.00 | 0.77 | 0.62 | 0.31 | 0.15 | 0.00 |
| E | 0.15 | 0.31 | 0.31 | 0.15 | 0.00 | 0.00 |
| ESE | 0.31 | 0.77 | 0.00 | 0.00 | 0.00 | 0.00 |
| SE | 0.00 | 0.46 | 0.15 | 0.77 | 0.00 | 0.00 |
| SSE | 0.15 | 0.31 | 0.77 | 0.77 | 0.15 | 1.08 |

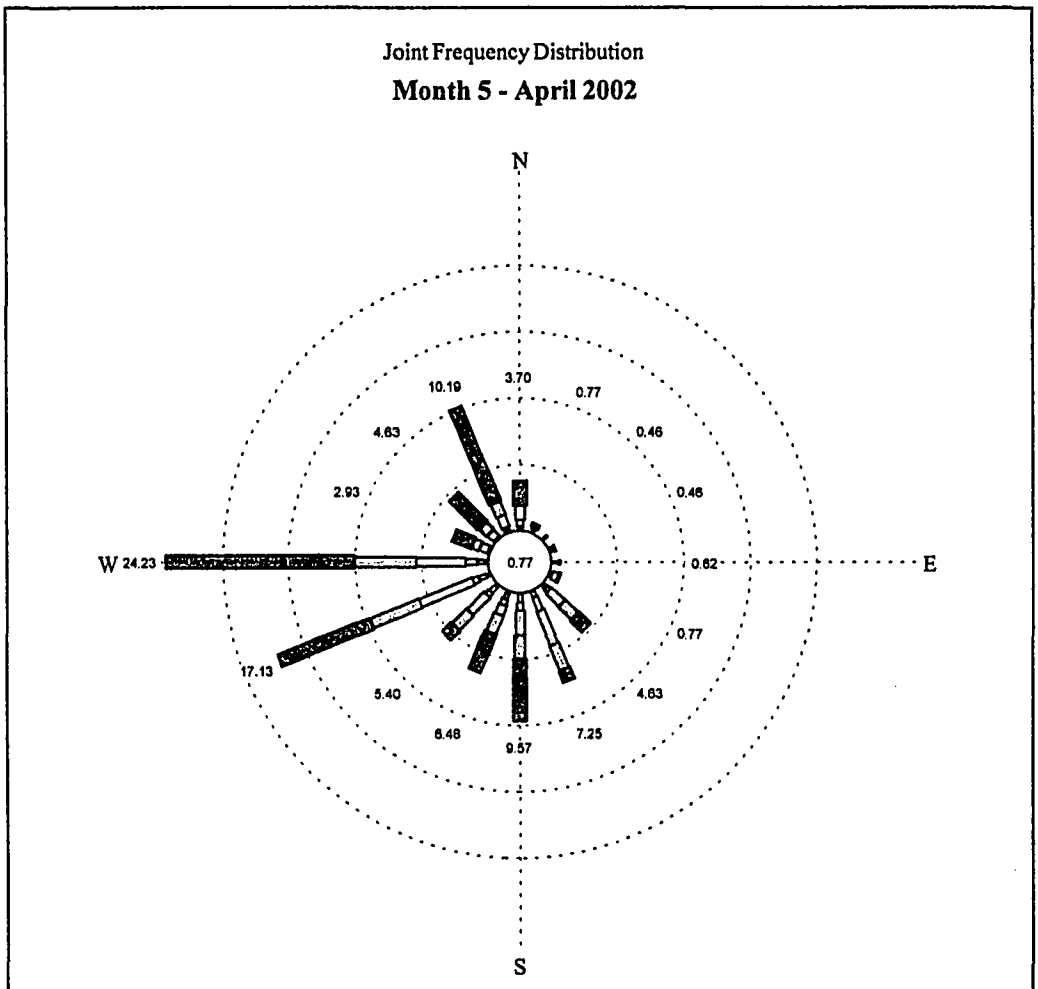
TOTAL OBS = 648 MISSING OBS = 0

PERCENT OCCURRENCE: Wind Speed ( Kilometers Per Hour)
LOWER BOUND OF CATEGORY

| DIR | 0 | 6 | 12 | 18 | 24 | 30 |
|---|---|---|---|---|---|---|
| S | 0.00 | 0.31 | 0.00 | 2.16 | 2.16 | 9.88 |
| SSW | 0.15 | 0.46 | 0.15 | 2.01 | 1.70 | 2.62 |
| SW | 0.15 | 0.62 | 1.08 | 1.54 | 1.39 | 2.31 |
| WSW | 0.00 | 0.93 | 1.39 | 1.39 | 3.40 | 3.70 |
| W | 0.31 | 2.78 | 4.48 | 3.86 | 5.09 | 6.48 |
| WNW | 0.15 | 0.62 | 0.77 | 1.08 | 1.54 | 1.85 |
| NW | 0.15 | 0.31 | 0.31 | 0.93 | 0.93 | 1.08 |
| NNW | 0.00 | 0.62 | 0.31 | 2.01 | 2.16 | 0.93 |

CALM OBS = 4 PERCENT CALM = 0.62

Figure III.4: Wind rose diagram for St. John's, Month 4 (10.02.2002 - 08.03.2002).




PERCENT OCCURRENCE: Wind Speed ( Kilometers Per Hour)
LOWER BOUND OF CATEGORY

| DIR | 0 | 6 | 12 | 18 | 24 | 30 |
|---|---|---|---|---|---|---|
| N | 0.15 | 0.15 | 0.46 | 1.08 | 0.00 | 1.85 |
| NNE | 0.31 | 0.00 | 0.15 | 0.31 | 0.00 | 0.00 |
| NE | 0.46 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| ENE | 0.00 | 0.31 | 0.15 | 0.00 | 0.00 | 0.00 |
| E | 0.46 | 0.15 | 0.00 | 0.00 | 0.00 | 0.00 |
| ESE | 0.00 | 0.15 | 0.00 | 0.46 | 0.15 | 0.00 |
| SE | 0.15 | 0.15 | 0.31 | 1.39 | 1.39 | 1.23 |
| SSE | 0.00 | 0.46 | 1.08 | 2.78 | 2.01 | 0.93 |

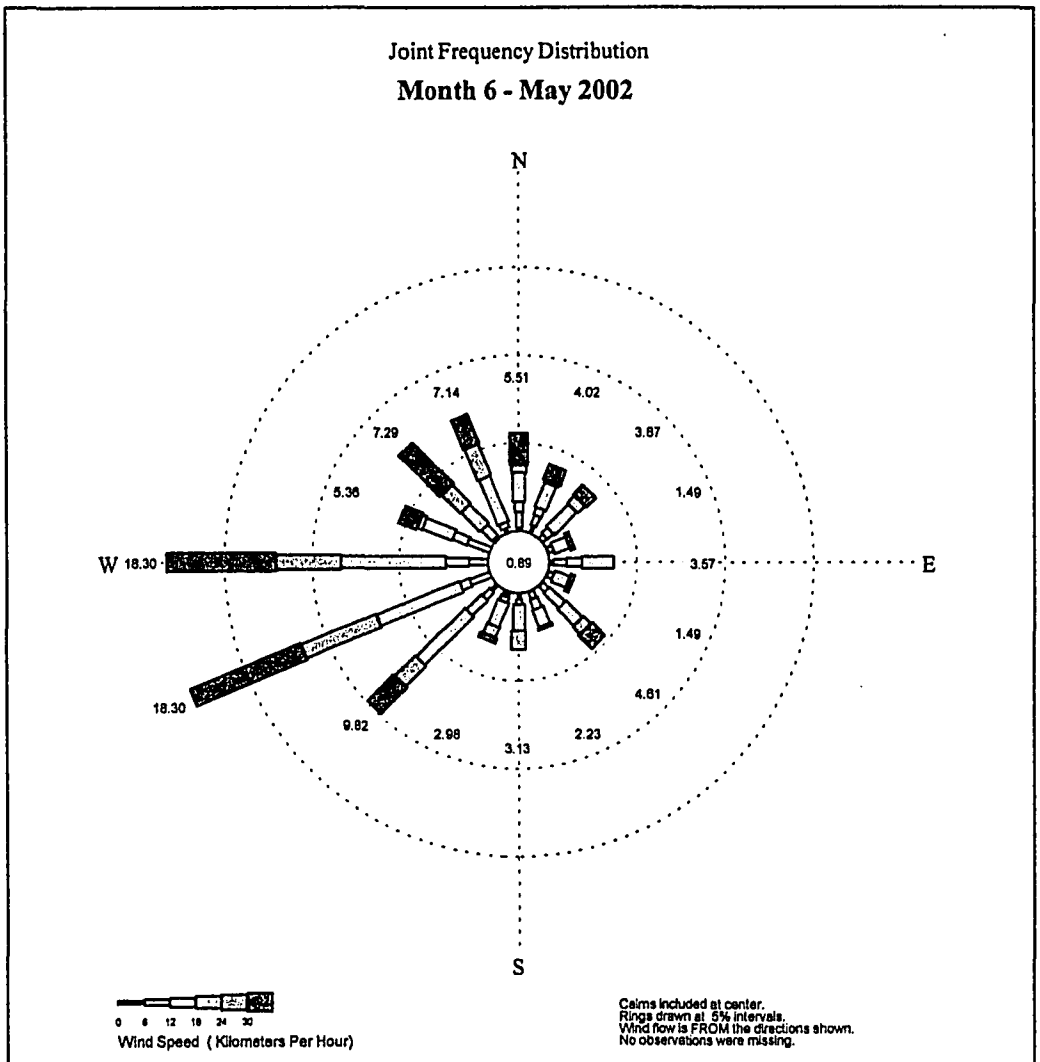
TOTAL OBS = 648 MISSING OBS = 0

PERCENT OCCURRENCE: Wind Speed ( Kilometers Per Hour)
LOWER BOUND OF CATEGORY

| DIR | 0 | 6 | 12 | 18 | 24 | 30 |
|---|---|---|---|---|---|---|
| S | 0.00 | 0.62 | 0.62 | 1.85 | 1.85 | 4.63 |
| SSW | 0.15 | 0.46 | 0.46 | 0.77 | 1.54 | 3.09 |
| SW | 0.15 | 0.62 | 0.31 | 1.85 | 1.54 | 0.93 |
| WSW | 0.31 | 0.62 | 0.46 | 4.32 | 4.01 | 7.41 |
| W | 0.15 | 0.62 | 0.93 | 3.70 | 4.63 | 14.20 |
| WNW | 0.15 | 0.00 | 0.00 | 0.62 | 0.62 | 1.54 |
| NW | 0.00 | 0.15 | 0.00 | 0.62 | 0.77 | 3.09 |
| NNW | 0.00 | 0.15 | 0.31 | 0.93 | 1.08 | 7.72 |

CALM OBS = 5 PERCENT CALM = 0.77

Figure III.5: Wind rose diagram for St. John's, Month 5 (10.03.2002 - 05.04.2002).




PERCENT OCCURRENCE: Wind Speed ( Kilometers Per Hour)
LOWER BOUND OF CATEGORY

| DIR | 0 | 6 | 12 | 18 | 24 | 30 |
|---|---|---|---|---|---|---|
| N | 0.15 | 0.89 | 0.60 | 1.64 | 0.45 | 1.79 |
| NNE | 0.45 | 0.60 | 0.45 | 1.49 | 0.15 | 0.89 |
| NE | 0.00 | 0.30 | 0.45 | 1.79 | 0.60 | 0.74 |
| ENE | 0.00 | 0.30 | 0.00 | 0.89 | 0.00 | 0.30 |
| E | 0.15 | 0.74 | 0.89 | 1.79 | 0.00 | 0.00 |
| ESE | 0.00 | 0.30 | 0.00 | 0.89 | 0.00 | 0.30 |
| SE | 0.00 | 0.45 | 1.19 | 1.34 | 0.60 | 1.04 |
| SSE | 0.00 | 0.45 | 0.60 | 1.04 | 0.15 | 0.00 |

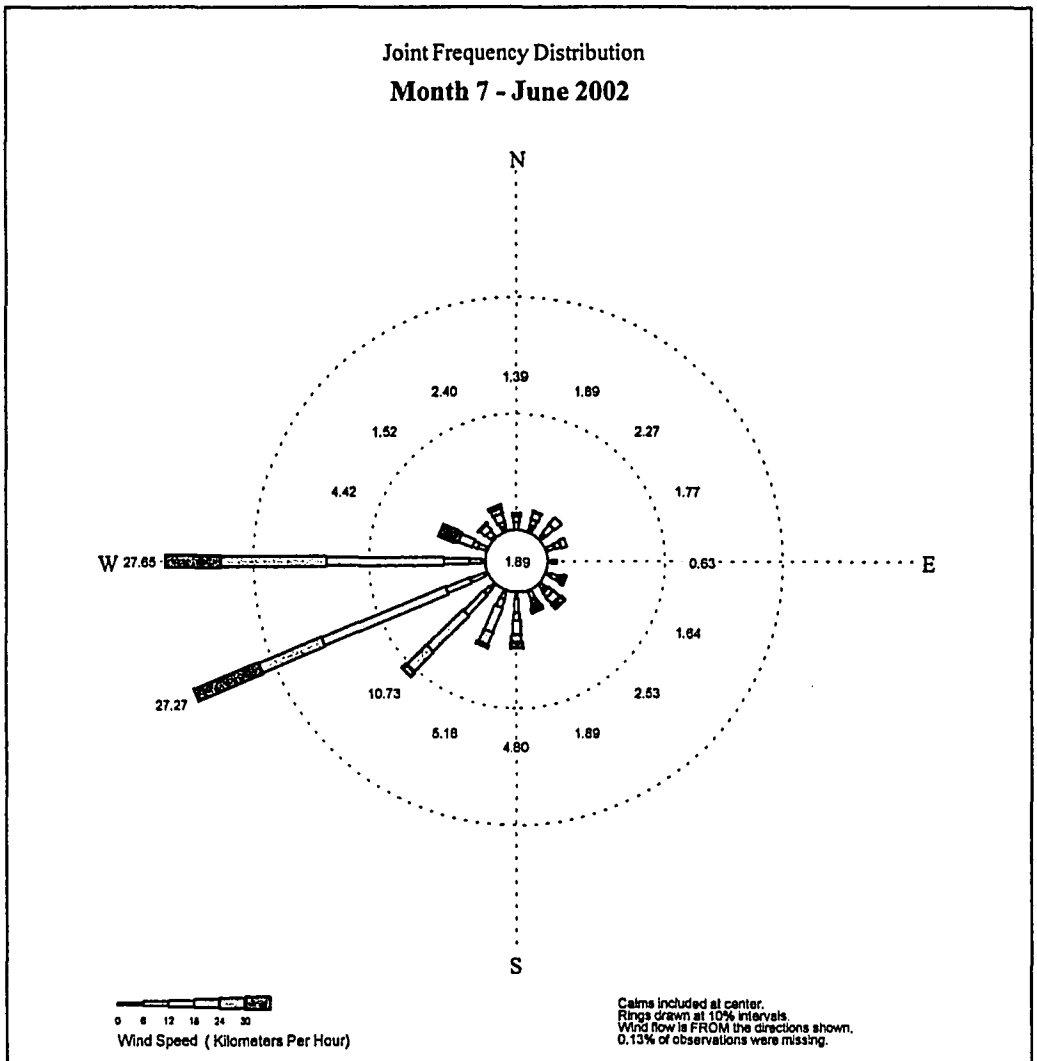
TOTAL OBS = 672 MISSING OBS = 0

PERCENT OCCURRENCE: Wind Speed ( Kilometers Per Hour)
LOWER BOUND OF CATEGORY

| DIR | 0 | 6 | 12 | 18 | 24 | 30 |
|---|---|---|---|---|---|---|
| S | 0.30 | 0.30 | 0.00 | 1.49 | 1.04 | 0.00 |
| SSW | 0.15 | 0.15 | 0.30 | 1.49 | 0.60 | 0.30 |
| SW | 0.45 | 0.60 | 1.19 | 3.87 | 1.64 | 2.08 |
| WSW | 0.00 | 1.19 | 0.60 | 5.06 | 4.61 | 6.85 |
| W | 0.00 | 1.04 | 1.34 | 5.95 | 3.72 | 6.25 |
| WNW | 0.00 | 1.34 | 0.89 | 1.79 | 0.30 | 1.04 |
| NW | 0.00 | 0.15 | 0.89 | 1.49 | 1.49 | 3.27 |
| NNW | 0.30 | 0.00 | 0.30 | 2.83 | 1.93 | 1.79 |

CALM OBS = 6 PERCENT CALM = 0.89

Figure III.6: Wind rose diagram for St. John's, Month 6 (07.04.2002 - 04.05.2002).




PERCENT OCCURRENCE: Wind Speed ( Kilometers Per Hour)
LOWER BOUND OF CATEGORY

| DIR | 0 | 6 | 12 | 18 | 24 | 30 |
|---|---|---|---|---|---|---|
| N | 0.00 | 0.63 | 0.51 | 0.25 | 0.00 | 0.00 |
| NNE | 0.25 | 0.38 | 0.51 | 0.63 | 0.13 | 0.00 |
| NE | 0.25 | 0.38 | 0.88 | 0.76 | 0.00 | 0.00 |
| ENE | 0.13 | 0.63 | 0.51 | 0.51 | 0.00 | 0.00 |
| E | 0.25 | 0.38 | 0.00 | 0.00 | 0.00 | 0.00 |
| ESE | 0.38 | 0.63 | 0.25 | 0.13 | 0.25 | 0.00 |
| SE | 0.00 | 0.51 | 0.25 | 0.51 | 0.76 | 0.51 |
| SSE | 0.00 | 0.76 | 0.38 | 0.25 | 0.13 | 0.38 |

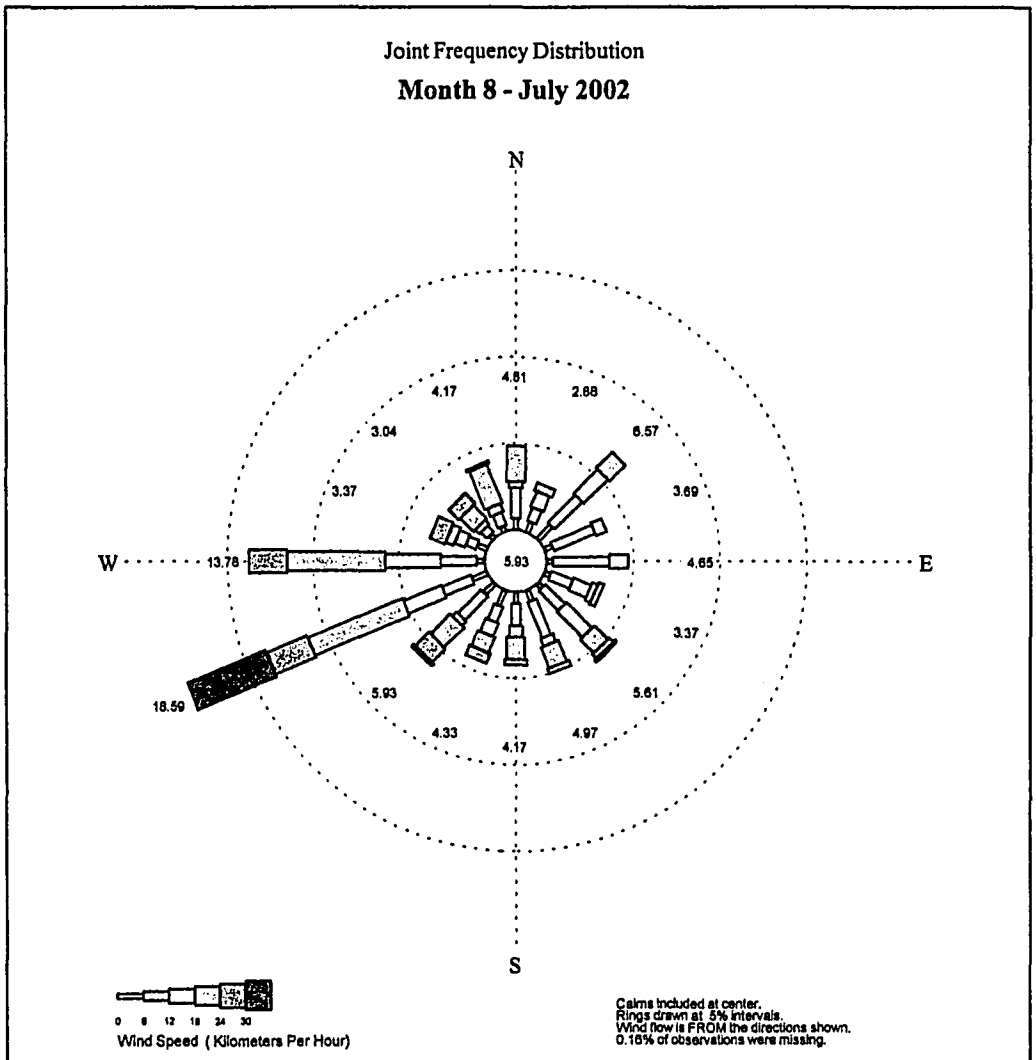
TOTAL OBS = 792 MISSING OBS = 1

PERCENT OCCURRENCE: Wind Speed ( Kilometers Per Hour)
LOWER BOUND OF CATEGORY

| DIR | 0 | 6 | 12 | 18 | 24 | 30 |
|---|---|---|---|---|---|---|
| S | 0.51 | 1.26 | 0.63 | 1.26 | 0.63 | 0.51 |
| SSW | 0.25 | 0.51 | 0.76 | 2.27 | 1.14 | 0.25 |
| SW | 0.51 | 0.63 | 2.40 | 4.29 | 2.40 | 0.51 |
| WSW | 0.25 | 1.39 | 2.15 | 11.49 | 5.93 | 6.06 |
| W | 0.25 | 1.14 | 2.15 | 10.10 | 9.22 | 4.80 |
| WNW | 0.13 | 0.76 | 0.51 | 1.26 | 0.38 | 1.39 |
| NW | 0.00 | 0.25 | 0.51 | 0.63 | 0.00 | 0.13 |
| NNW | 0.13 | 0.38 | 0.38 | 0.76 | 0.51 | 0.25 |

CALM OBS = 15 PERCENT CALM = 1.89

Figure III.7: Wind rose diagram for St. John's, Month 7 (06.05.2002 - 07.06.2002).




PERCENT OCCURRENCE: Wind Speed ( Kilometers Per Hour)
LOWER BOUND OF CATEGORY

| DIR | 0 | 6 | 12 | 18 | 24 | 30 |
|---|---|---|---|---|---|---|
| N | 0.64 | 1.76 | 0.32 | 2.08 | 0.00 | 0.00 |
| NNE | 0.64 | 0.80 | 0.96 | 0.48 | 0.00 | 0.00 |
| NE | 1.12 | 2.24 | 1.92 | 1.28 | 0.00 | 0.00 |
| ENE | 0.48 | 2.56 | 0.64 | 0.00 | 0.00 | 0.00 |
| E | 0.32 | 3.21 | 1.12 | 0.00 | 0.00 | 0.00 |
| ESE | 0.32 | 1.28 | 1.12 | 0.32 | 0.32 | 0.00 |
| SE | 0.32 | 1.60 | 2.08 | 1.12 | 0.32 | 0.16 |
| SSE | 0.64 | 2.24 | 0.48 | 1.28 | 0.32 | 0.00 |

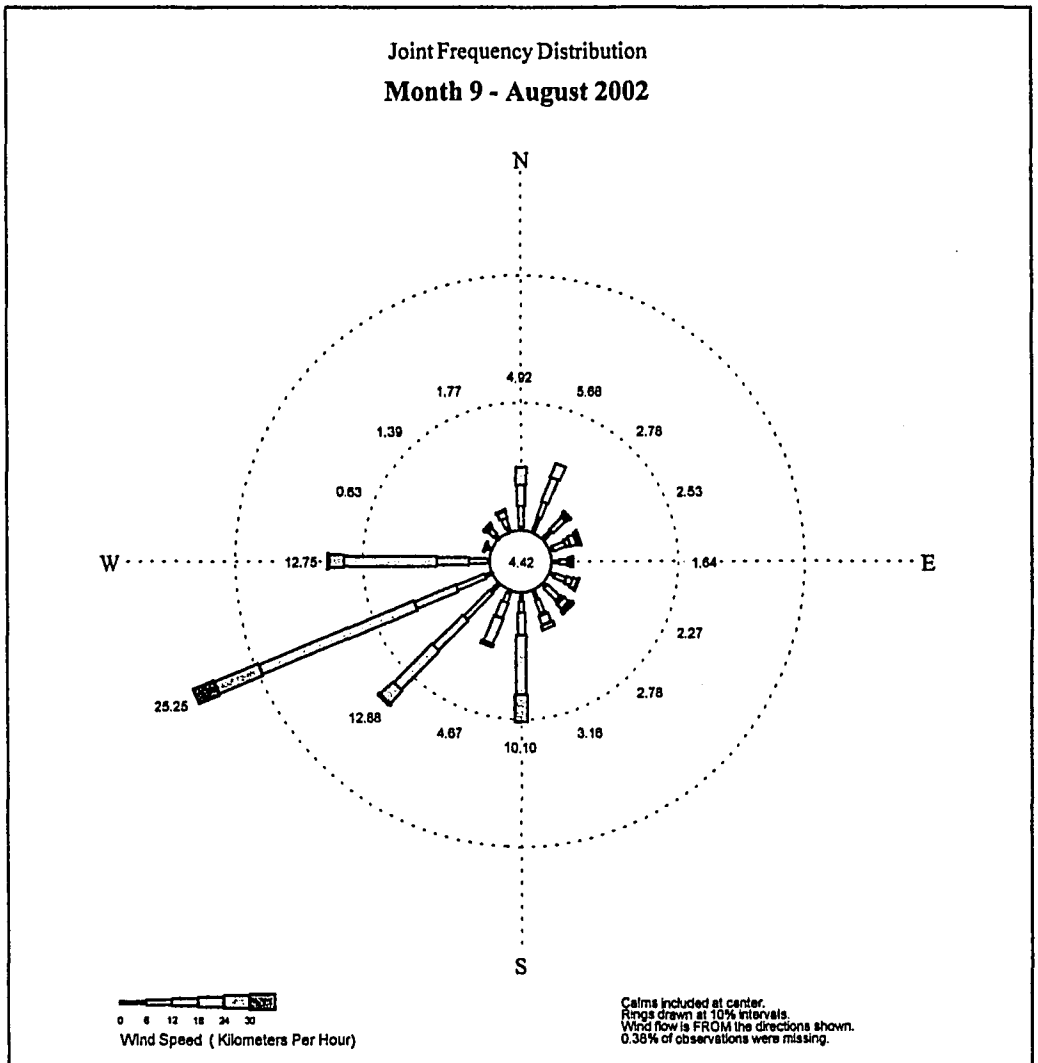
TOTAL OBS = 624 MISSING OBS = 1

PERCENT OCCURRENCE: Wind Speed ( Kilometers Per Hour)
LOWER BOUND OF CATEGORY

| DIR | 0 | 6 | 12 | 18 | 24 | 30 |
|---|---|---|---|---|---|---|
| S | 0.64 | 1.28 | 0.64 | 1.28 | 0.32 | 0.00 |
| SSW | 0.96 | 0.96 | 0.80 | 0.96 | 0.64 | 0.00 |
| SW | 0.80 | 1.92 | 0.32 | 1.60 | 1.12 | 0.16 |
| WSW | 0.96 | 1.76 | 2.40 | 5.93 | 2.56 | 4.97 |
| W | 0.48 | 2.08 | 3.21 | 5.77 | 2.24 | 0.00 |
| WNW | 0.64 | 0.64 | 0.64 | 0.48 | 0.96 | 0.00 |
| NW | 0.32 | 0.48 | 0.32 | 1.12 | 0.80 | 0.00 |
| NNW | 0.32 | 0.80 | 0.48 | 2.40 | 0.16 | 0.00 |

CALM OBS = 37 PERCENT CALM = 5.93

Figure III.8: Wind rose diagram for St. John's, Month 8 (10.06.2002 - 05.07.2002).




PERCENT OCCURRENCE: Wind Speed ( Kilometers Per Hour)
LOWER BOUND OF CATEGORY

| DIR | 0 | 6 | 12 | 18 | 24 | 30 |
|---|---|---|---|---|---|---|
| N | 0.25 | 1.64 | 1.64 | 1.39 | 0.00 | 0.00 |
| NNE | 1.26 | 1.26 | 2.02 | 1.14 | 0.00 | 0.00 |
| NE | 0.88 | 1.39 | 0.25 | 0.25 | 0.00 | 0.00 |
| ENE | 0.25 | 1.01 | 0.38 | 0.51 | 0.25 | 0.13 |
| E | 0.51 | 0.63 | 0.25 | 0.25 | 0.00 | 0.00 |
| ESE | 0.38 | 0.88 | 0.51 | 0.38 | 0.13 | 0.00 |
| SE | 0.25 | 1.39 | 0.25 | 0.51 | 0.13 | 0.25 |
| SSE | 0.76 | 1.14 | 0.13 | 0.76 | 0.38 | 0.00 |

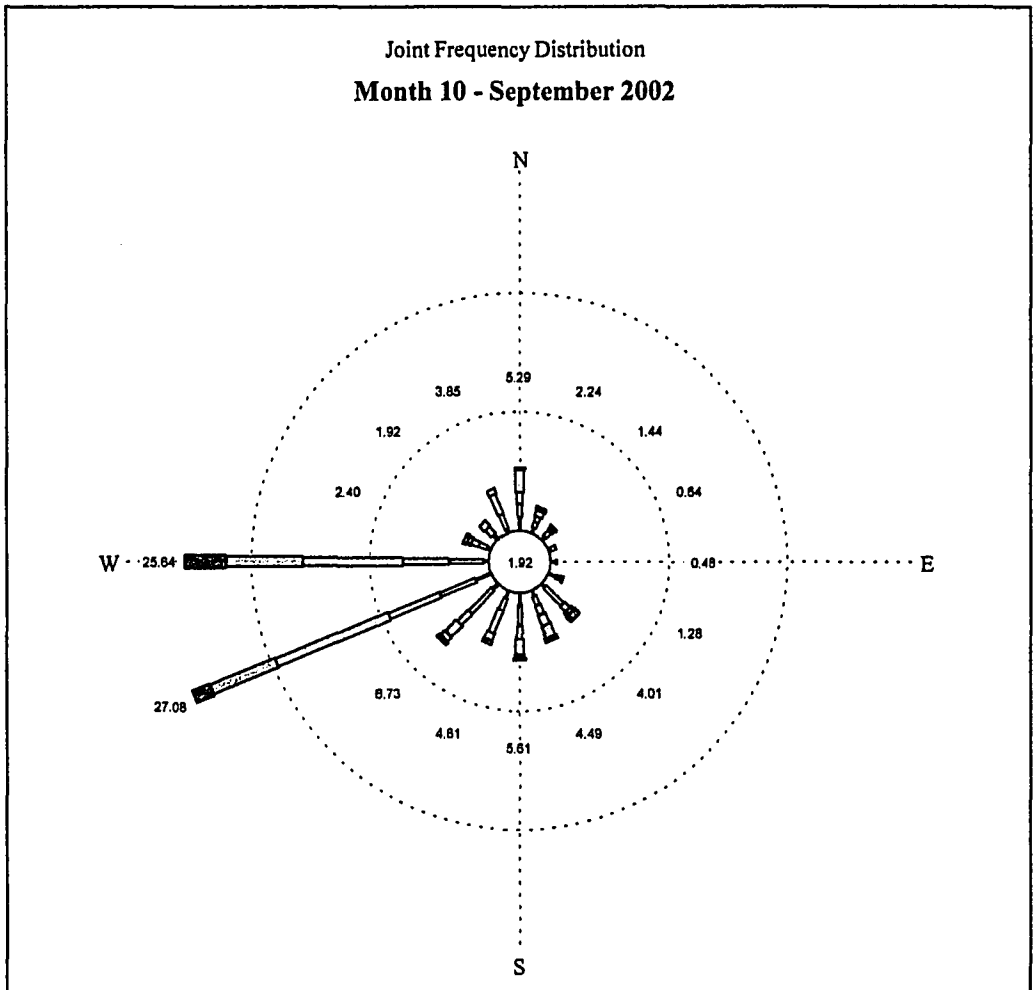
TOTAL OBS = 792 MISSING OBS = 3

PERCENT OCCURRENCE: Wind Speed ( Kilometers Per Hour)
LOWER BOUND OF CATEGORY

| DIR | 0 | 6 | 12 | 18 | 24 | 30 |
|---|---|---|---|---|---|---|
| S | 0.63 | 0.88 | 1.77 | 4.67 | 2.15 | 0.00 |
| SSW | 0.25 | 0.76 | 1.26 | 2.15 | 0.25 | 0.00 |
| SW | 0.88 | 2.90 | 3.28 | 4.29 | 1.39 | 0.13 |
| WSW | 0.63 | 2.40 | 3.54 | 13.13 | 3.79 | 1.77 |
| W | 0.13 | 1.52 | 2.53 | 7.32 | 1.14 | 0.13 |
| WNW | 0.38 | 0.13 | 0.00 | 0.13 | 0.00 | 0.00 |
| NW | 0.38 | 0.63 | 0.25 | 0.13 | 0.00 | 0.00 |
| NNW | 0.38 | 0.76 | 0.51 | 0.13 | 0.00 | 0.00 |

CALM OBS = 35 PERCENT CALM = 4.42

Figure III.9: Wind rose diagram for St. John's, Month 9 (07.07.2002 - 08.08.2002).




PERCENT OCCURRENCE: Wind Speed ( Kilometers Per Hour)
LOWER BOUND OF CATEGORY

| DIR | 0 | 6 | 12 | 18 | 24 | 30 |
|---|---|---|---|---|---|---|
| N | 1.12 | 1.12 | 0.96 | 1.92 | 0.16 | 0.00 |
| NNE | 0.48 | 0.48 | 0.48 | 0.48 | 0.32 | 0.00 |
| NE | 0.32 | 0.64 | 0.32 | 0.16 | 0.00 | 0.00 |
| ENE | 0.16 | 0.48 | 0.00 | 0.00 | 0.00 | 0.00 |
| E | 0.32 | 0.16 | 0.00 | 0.00 | 0.00 | 0.00 |
| ESE | 0.64 | 0.48 | 0.16 | 0.00 | 0.00 | 0.00 |
| SE | 0.32 | 2.08 | 0.64 | 0.00 | 0.32 | 0.64 |
| SSE | 0.48 | 0.80 | 0.80 | 0.96 | 1.12 | 0.32 |

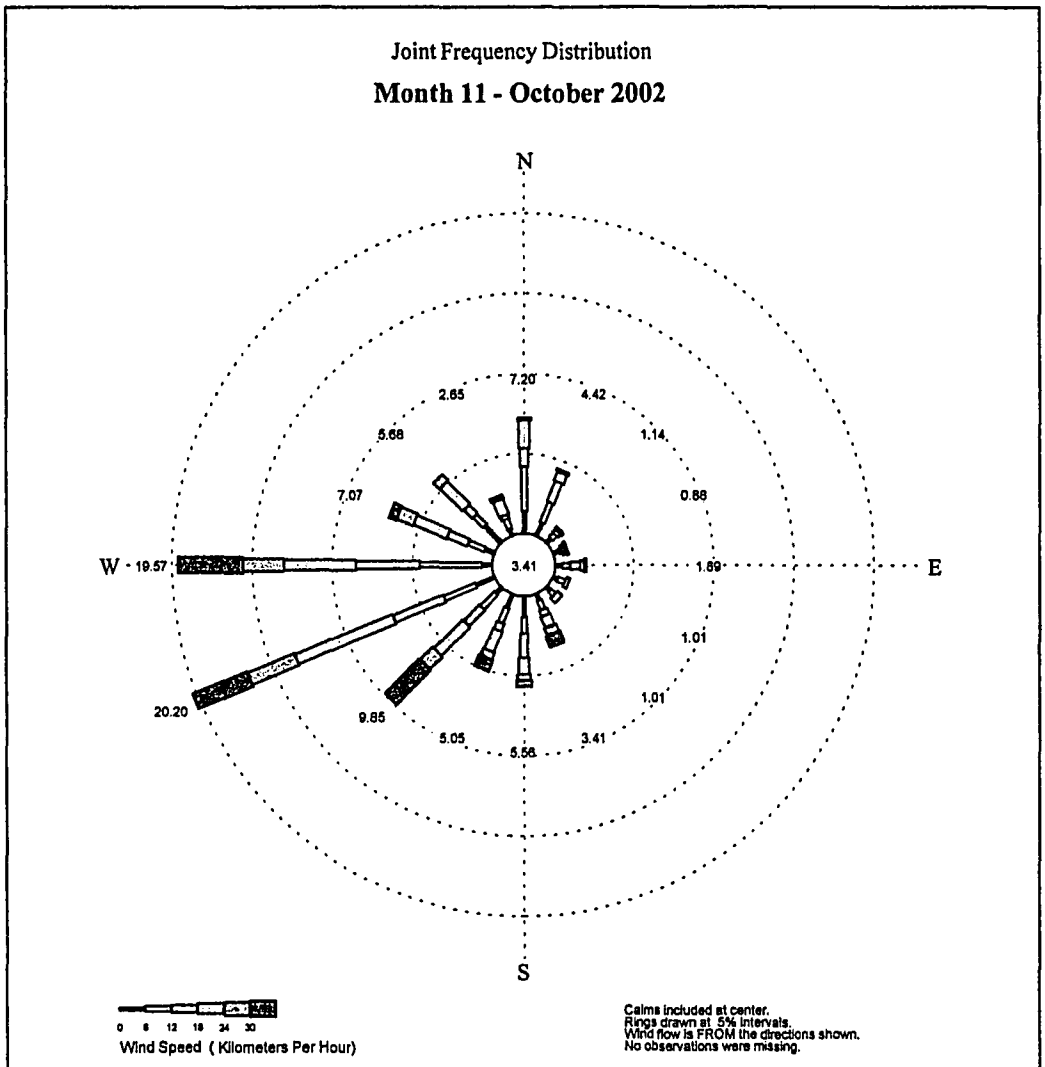
TOTAL OBS = 624 MISSING OBS = 1

PERCENT OCCURRENCE: Wind Speed ( Kilometers Per Hour)
LOWER BOUND OF CATEGORY

| DIR | 0 | 6 | 12 | 18 | 24 | 30 |
|---|---|---|---|---|---|---|
| S | 0.96 | 1.76 | 1.12 | 1.28 | 0.32 | 0.16 |
| SSW | 0.64 | 1.12 | 2.08 | 0.64 | 0.32 | 0.00 |
| SW | 0.64 | 2.72 | 1.28 | 1.28 | 0.48 | 0.32 |
| WSW | 1.44 | 3.21 | 4.65 | 10.26 | 5.93 | 1.60 |
| W | 0.48 | 2.88 | 3.85 | 8.49 | 6.41 | 3.53 |
| WNW | 0.32 | 0.80 | 0.64 | 0.32 | 0.32 | 0.00 |
| NW | 0.48 | 0.00 | 0.80 | 0.64 | 0.00 | 0.00 |
| NNW | 0.32 | 1.28 | 1.76 | 0.48 | 0.00 | 0.00 |

CALM OBS = 12 PERCENT CALM = 1.92

Figure III.10: Wind rose diagram for St. John's, Month 10 (11.08.2002 - 05.09.2002).




PERCENT OCCURRENCE: Wind Speed ( Kilometers Per Hour)
LOWER BOUND OF CATEGORY

| DIR | 0 | 6 | 12 | 18 | 24 | 30 |
|---|---|---|---|---|---|---|
| N | 1.39 | 2.78 | 1.14 | 1.77 | 0.13 | 0.00 |
| NNE | 0.88 | 1.39 | 1.39 | 0.63 | 0.13 | 0.00 |
| NE | 0.38 | 0.38 | 0.25 | 0.13 | 0.00 | 0.00 |
| ENE | 0.38 | 0.13 | 0.00 | 0.25 | 0.13 | 0.00 |
| E | 0.38 | 0.51 | 0.63 | 0.25 | 0.13 | 0.00 |
| ESE | 0.25 | 0.51 | 0.00 | 0.25 | 0.00 | 0.00 |
| SE | 0.25 | 0.38 | 0.00 | 0.38 | 0.00 | 0.00 |
| SSE | 0.38 | 0.63 | 0.38 | 0.76 | 0.51 | 0.76 |

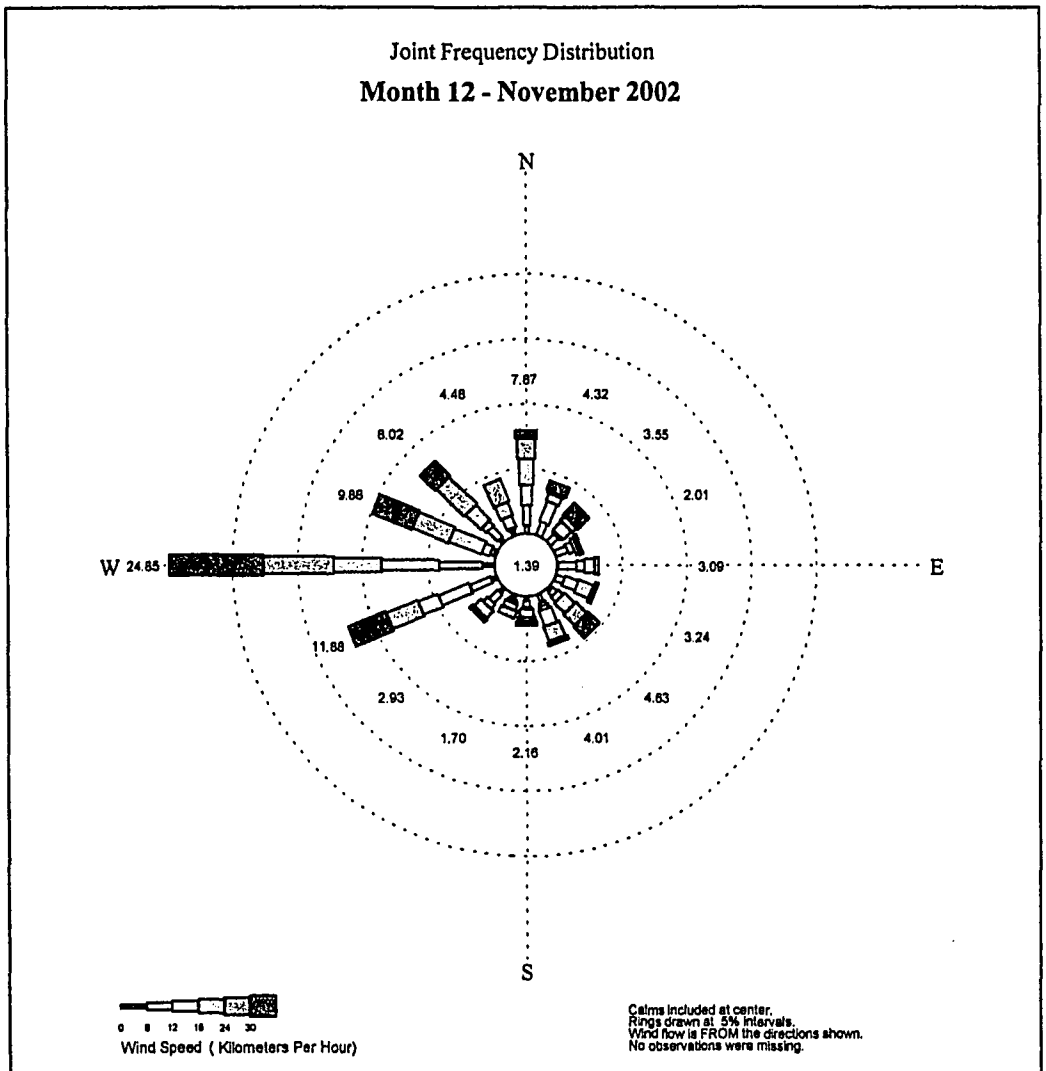
TOTAL OBS = 792 MISSING OBS = 0

PERCENT OCCURRENCE: Wind Speed ( Kilometers Per Hour)
LOWER BOUND OF CATEGORY

| DIR | 0 | 6 | 12 | 18 | 24 | 30 |
|---|---|---|---|---|---|---|
| S | 1.26 | 1.01 | 1.52 | 1.01 | 0.38 | 0.38 |
| SSW | 0.88 | 1.01 | 0.51 | 1.39 | 0.51 | 0.76 |
| SW | 0.51 | 1.26 | 1.39 | 2.53 | 1.14 | 3.03 |
| WSW | 1.26 | 2.15 | 3.41 | 6.57 | 3.16 | 3.66 |
| W | 0.63 | 3.91 | 3.91 | 4.55 | 2.53 | 4.04 |
| WNW | 0.63 | 1.14 | 1.39 | 2.27 | 1.14 | 0.51 |
| NW | 1.52 | 0.88 | 0.76 | 2.02 | 0.51 | 0.00 |
| NNW | 0.51 | 0.63 | 0.25 | 1.01 | 0.25 | 0.00 |

CALM OBS = 27 PERCENT CALM = 3.41

Figure III.11: Wind rose diagram for St. John's, Month 11 (08.09.2002 - 10.10.2002).




PERCENT OCCURRENCE: Wind Speed ( Kilometers Per Hour)
LOWER BOUND OF CATEGORY

| DIR | 0 | 6 | 12 | 18 | 24 | 30 |
|---|---|---|---|---|---|---|
| N | 0.62 | 1.54 | 1.54 | 2.01 | 1.54 | 0.62 |
| NNE | 0.00 | 1.08 | 1.70 | 0.62 | 0.15 | 0.77 |
| NE | 0.00 | 0.77 | 0.15 | 0.93 | 0.46 | 1.23 |
| ENE | 0.00 | 0.93 | 0.46 | 0.00 | 0.31 | 0.31 |
| E | 0.00 | 1.39 | 0.62 | 0.77 | 0.31 | 0.00 |
| ESE | 0.00 | 0.62 | 1.08 | 1.23 | 0.00 | 0.31 |
| SE | 0.15 | 0.46 | 0.46 | 1.08 | 1.23 | 1.23 |
| SSE | 0.62 | 0.31 | 0.31 | 1.23 | 1.23 | 0.31 |

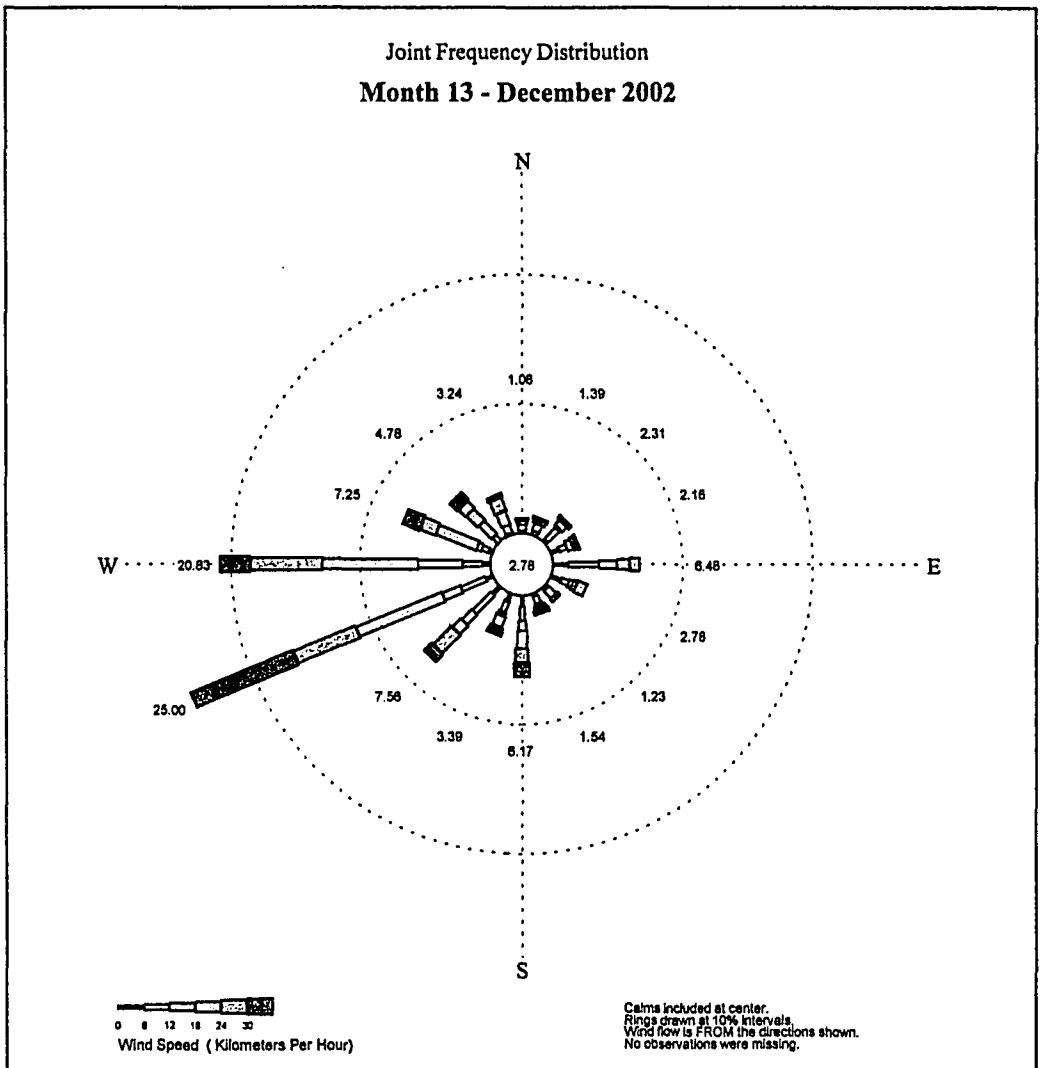
TOTAL OBS = 648 MISSING OBS = 0

PERCENT OCCURRENCE: Wind Speed ( Kilometers Per Hour)
LOWER BOUND OF CATEGORY

| DIR | 0 | 6 | 12 | 18 | 24 | 30 |
|---|---|---|---|---|---|---|
| S | 0.31 | 0.46 | 0.15 | 0.62 | 0.31 | 0.31 |
| SSW | 0.31 | 0.15 | 0.15 | 0.46 | 0.62 | 0.00 |
| SW | 0.46 | 0.93 | 0.46 | 0.00 | 0.77 | 0.31 |
| WSW | 0.46 | 1.70 | 2.47 | 1.54 | 2.62 | 3.09 |
| W | 0.93 | 3.24 | 4.32 | 3.70 | 5.40 | 7.25 |
| WNW | 0.15 | 0.31 | 0.62 | 2.62 | 2.93 | 3.24 |
| NW | 0.46 | 1.08 | 0.46 | 1.54 | 2.93 | 1.54 |
| NNW | 0.46 | 0.15 | 0.93 | 1.08 | 1.85 | 0.00 |

CALM OBS = 9 PERCENT CALM = 1.39

Figure III.12: Wind rose diagram for St. John's, Month 12 (13.10.2002 - 08.11.2002).




PERCENT OCCURRENCE: Wind Speed ( Kilometers Per Hour)
LOWER BOUND OF CATEGORY

| DIR | 0 | 6 | 12 | 18 | 24 | 30 |
|---|---|---|---|---|---|---|
| N | 0.15 | 0.00 | 0.46 | 0.31 | 0.15 | 0.00 |
| NNE | 0.00 | 0.31 | 0.62 | 0.00 | 0.31 | 0.15 |
| NE | 0.15 | 1.23 | 0.15 | 0.46 | 0.00 | 0.31 |
| ENE | 0.62 | 0.62 | 0.00 | 0.46 | 0.31 | 0.15 |
| E | 1.08 | 2.31 | 1.39 | 0.93 | 0.77 | 0.00 |
| ESE | 0.77 | 0.62 | 0.31 | 0.31 | 0.77 | 0.00 |
| SE | 0.00 | 0.15 | 0.77 | 0.31 | 0.00 | 0.00 |
| SSE | 0.15 | 0.62 | 0.15 | 0.15 | 0.15 | 0.31 |

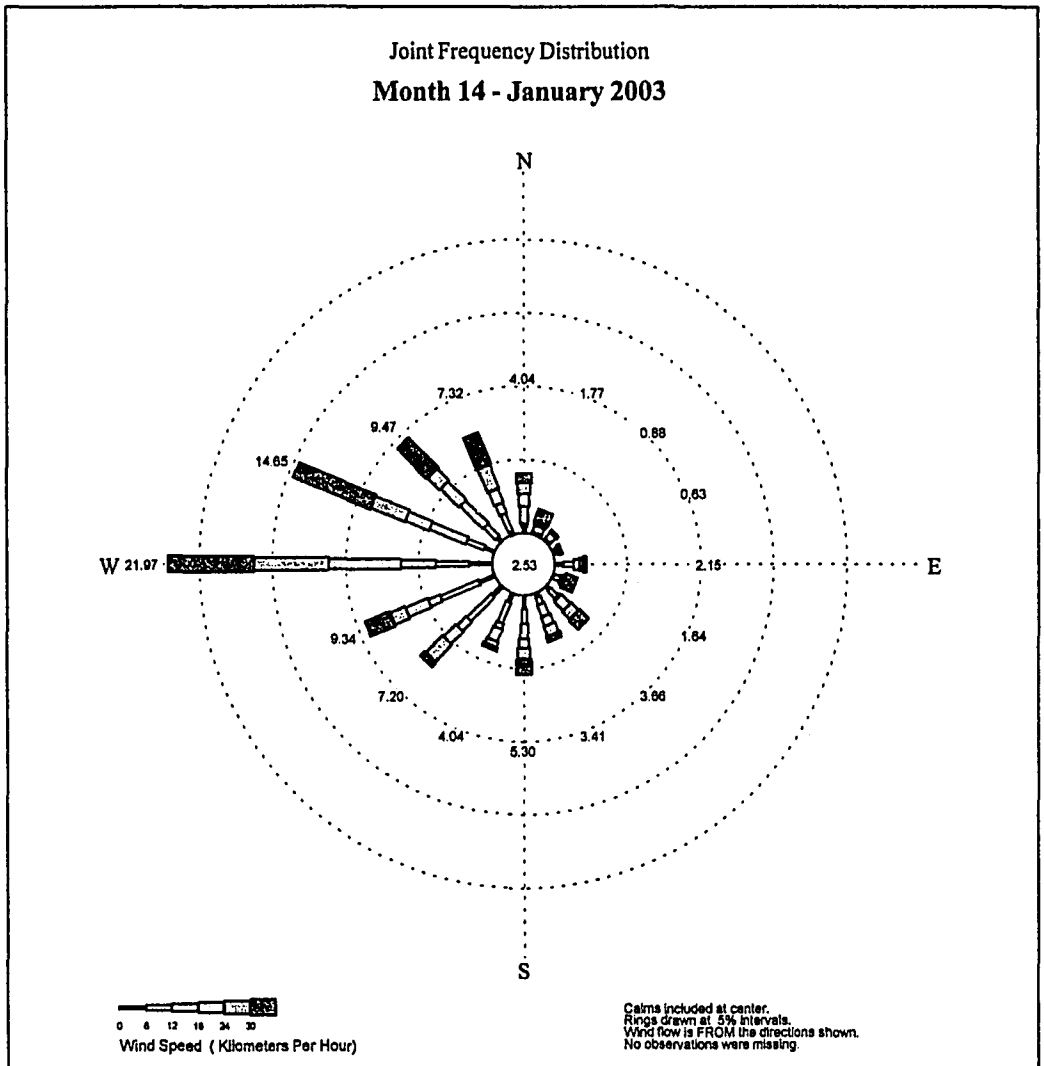
TOTAL OBS = 648 MISSING OBS = 0

PERCENT OCCURRENCE: Wind Speed ( Kilometers Per Hour)
LOWER BOUND OF CATEGORY

| DIR | 0 | 6 | 12 | 18 | 24 | 30 |
|---|---|---|---|---|---|---|
| S | 0.77 | 1.08 | 0.77 | 1.39 | 1.08 | 1.08 |
| SSW | 0.62 | 0.93 | 0.31 | 0.93 | 0.31 | 0.31 |
| SW | 0.77 | 2.01 | 1.08 | 0.93 | 2.01 | 0.77 |
| WSW | 0.62 | 2.01 | 1.54 | 7.10 | 5.25 | 8.49 |
| W | 0.62 | 1.54 | 3.40 | 7.41 | 5.56 | 2.31 |
| WNW | 0.31 | 0.62 | 0.31 | 3.40 | 1.23 | 1.39 |
| NW | 0.00 | 0.62 | 1.39 | 1.23 | 0.77 | 0.77 |
| NNW | 0.15 | 0.00 | 0.62 | 1.08 | 1.08 | 0.31 |

CALM OBS = 18 PERCENT CALM = 2.78

Figure III.13: Wind rose diagram for St. John's, Month 13 (10.11.2002 - 06.12.2002).




PERCENT OCCURRENCE: Wind Speed ( Kilometers Per Hour)
LOWER BOUND OF CATEGORY

| DIR | 0 | 6 | 12 | 18 | 24 | 30 |
|---|---|---|---|---|---|---|
| N | 0.38 | 0.25 | 1.14 | 0.88 | 0.76 | 0.63 |
| NNE | 0.00 | 0.00 | 0.13 | 0.51 | 0.25 | 0.88 |
| NE | 0.13 | 0.00 | 0.51 | 0.25 | 0.00 | 0.00 |
| ENE | 0.13 | 0.13 | 0.13 | 0.25 | 0.00 | 0.00 |
| E | 0.51 | 0.76 | 0.00 | 0.38 | 0.25 | 0.25 |
| ESE | 0.00 | 0.51 | 0.13 | 0.13 | 0.00 | 0.88 |
| SE | 0.25 | 0.76 | 0.13 | 0.88 | 0.76 | 0.88 |
| SSE | 0.25 | 0.51 | 0.88 | 0.63 | 0.76 | 0.38 |

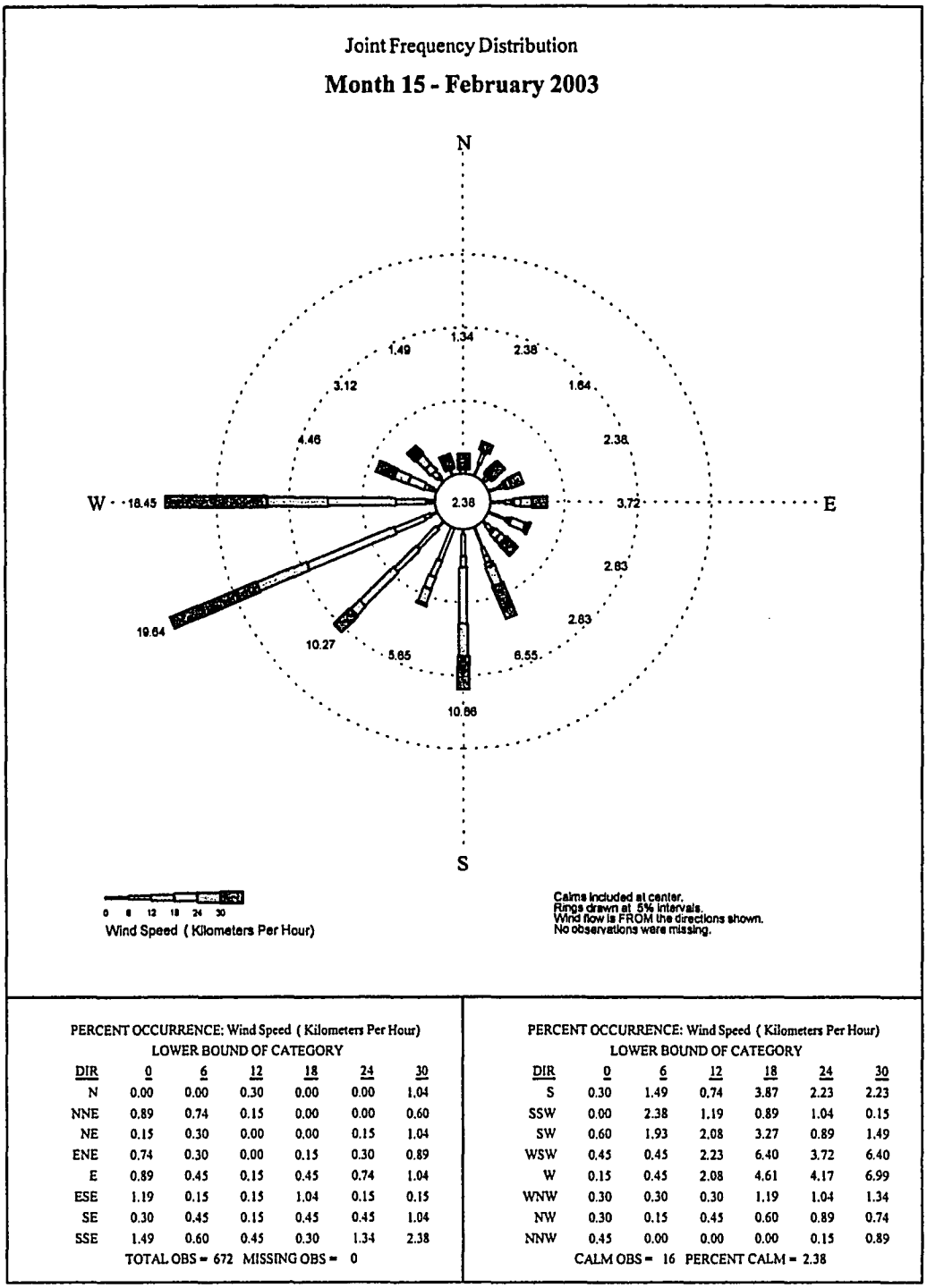
TOTAL OBS = 792 MISSING OBS = 0

PERCENT OCCURRENCE: Wind Speed ( Kilometers Per Hour)
LOWER BOUND OF CATEGORY

| DIR | 0 | 6 | 12 | 18 | 24 | 30 |
|---|---|---|---|---|---|---|
| S | 0.88 | 1.01 | 1.01 | 0.63 | 0.76 | 1.01 |
| SSW | 0.63 | 1.52 | 0.38 | 0.88 | 0.38 | 0.25 |
| SW | 0.88 | 2.27 | 0.88 | 1.14 | 1.64 | 0.38 |
| WSW | 1.14 | 2.78 | 0.88 | 1.64 | 1.14 | 1.77 |
| W | 1.52 | 2.27 | 2.40 | 4.92 | 5.05 | 5.81 |
| WNW | 0.76 | 1.26 | 2.65 | 1.77 | 2.53 | 5.68 |
| NW | 0.38 | 1.14 | 2.15 | 1.77 | 1.14 | 2.90 |
| NNW | 0.25 | 1.26 | 0.76 | 1.01 | 1.77 | 2.27 |

CALM OBS = 20 PERCENT CALM = 2.53

Figure III.14: Wind rose diagram for St. John's, Month 14 (09.12.2002 - 10.01.2003).




PERCENT OCCURRENCE: Wind Speed ( Kilometers Per Hour)
LOWER BOUND OF CATEGORY

| DIR | 0 | 6 | 12 | 18 | 24 | 30 |
|---|---|---|---|---|---|---|
| N | 0.00 | 0.00 | 0.30 | 0.00 | 0.00 | 1.04 |
| NNE | 0.89 | 0.74 | 0.15 | 0.00 | 0.00 | 0.60 |
| NE | 0.15 | 0.30 | 0.00 | 0.00 | 0.15 | 1.04 |
| ENE | 0.74 | 0.30 | 0.00 | 0.15 | 0.30 | 0.89 |
| E | 0.89 | 0.45 | 0.15 | 0.45 | 0.74 | 1.04 |
| ESE | 1.19 | 0.15 | 0.15 | 1.04 | 0.15 | 0.15 |
| SE | 0.30 | 0.45 | 0.15 | 0.45 | 0.45 | 1.04 |
| SSE | 1.49 | 0.60 | 0.45 | 0.30 | 1.34 | 2.38 |

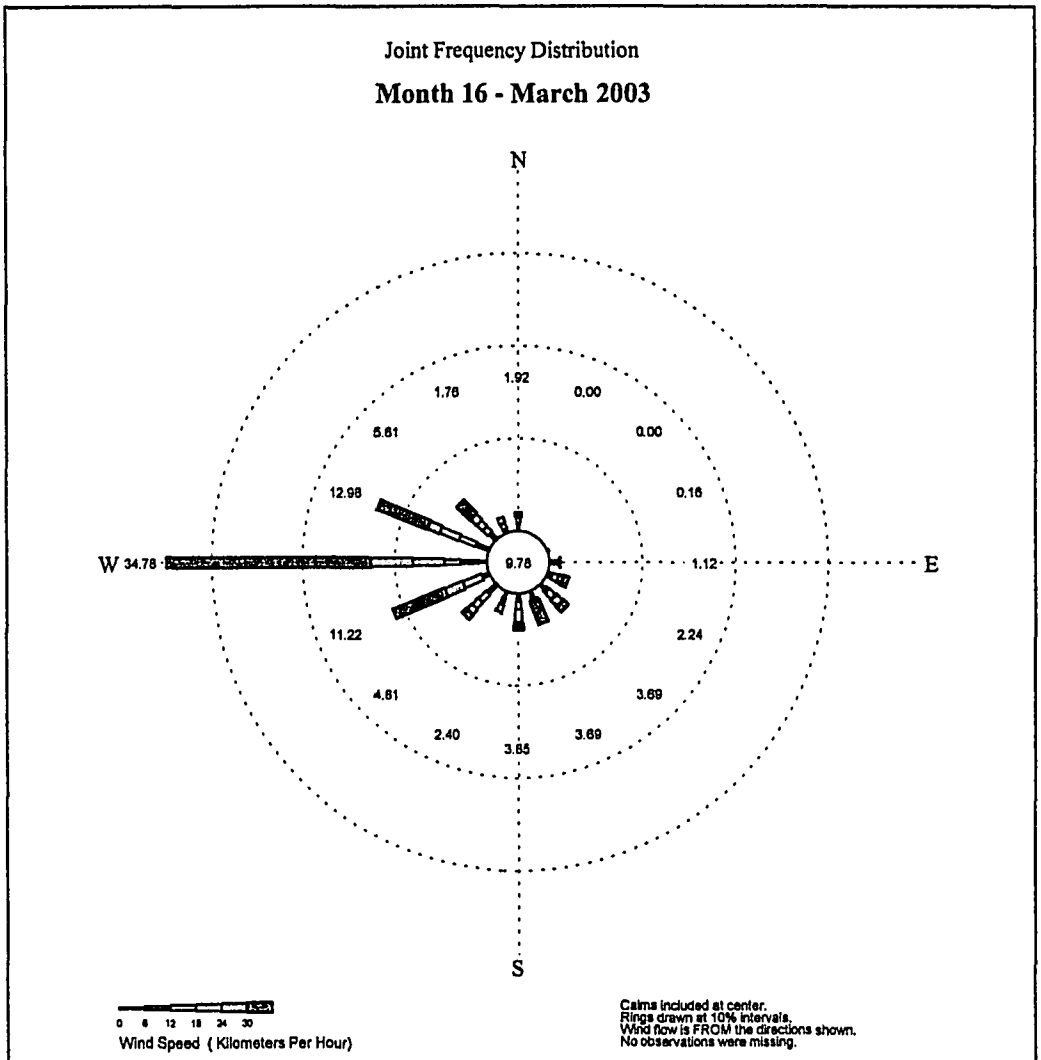
TOTAL OBS = 672 MISSING OBS = 0

PERCENT OCCURRENCE: Wind Speed ( Kilometers Per Hour)
LOWER BOUND OF CATEGORY

| DIR | 0 | 6 | 12 | 18 | 24 | 30 |
|---|---|---|---|---|---|---|
| S | 0.30 | 1.49 | 0.74 | 3.87 | 2.23 | 2.23 |
| SSW | 0.00 | 2.38 | 1.19 | 0.89 | 1.04 | 0.15 |
| SW | 0.60 | 1.93 | 2.08 | 3.27 | 0.89 | 1.49 |
| WSW | 0.45 | 0.45 | 2.23 | 6.40 | 3.72 | 6.40 |
| W | 0.15 | 0.45 | 2.08 | 4.61 | 4.17 | 6.99 |
| WNW | 0.30 | 0.30 | 0.30 | 1.19 | 1.04 | 1.34 |
| NW | 0.30 | 0.15 | 0.45 | 0.60 | 0.89 | 0.74 |
| NNW | 0.45 | 0.00 | 0.00 | 0.00 | 0.15 | 0.89 |

CALM OBS = 16 PERCENT CALM = 2.38

Figure III.15: Wind rose diagram for St. John's, Month 15 (12.01.2003 - 08.02.2003).




PERCENT OCCURRENCE: Wind Speed ( Kilometers Per Hour)
LOWER BOUND OF CATEGORY

| DIR | 0 | 6 | 12 | 18 | 24 | 30 |
|---|---|---|---|---|---|---|
| N | 0.80 | 0.64 | 0.16 | 0.32 | 0.00 | 0.00 |
| NNE | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| NE | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| ENE | 0.16 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| E | 0.00 | 0.80 | 0.16 | 0.00 | 0.00 | 0.16 |
| ESE | 0.00 | 0.16 | 0.32 | 0.64 | 0.48 | 0.64 |
| SE | 0.48 | 0.16 | 0.32 | 0.96 | 0.96 | 0.80 |
| SSE | 0.32 | 0.48 | 0.00 | 0.16 | 0.16 | 2.56 |

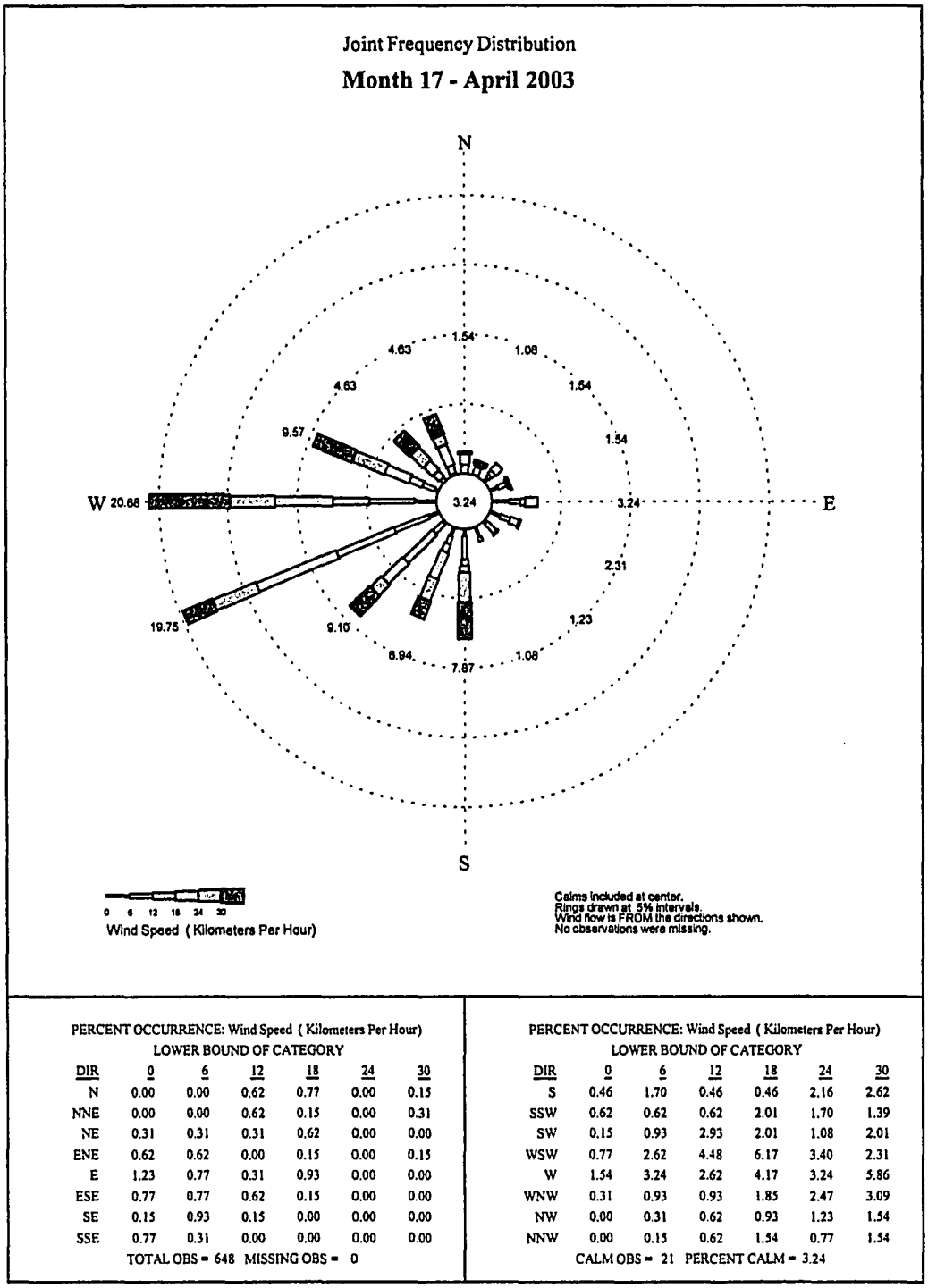
TOTAL OBS = 624 MISSING OBS = 0

PERCENT OCCURRENCE: Wind Speed ( Kilometers Per Hour)
LOWER BOUND OF CATEGORY

| DIR | 0 | 6 | 12 | 18 | 24 | 30 |
|---|---|---|---|---|---|---|
| S | 0.16 | 0.64 | 0.80 | 1.44 | 0.32 | 0.48 |
| SSW | 0.64 | 0.80 | 0.80 | 0.16 | 0.00 | 0.00 |
| SW | 0.16 | 1.12 | 1.12 | 0.96 | 0.80 | 0.64 |
| WSW | 0.32 | 0.48 | 0.00 | 2.24 | 2.08 | 6.09 |
| W | 0.80 | 2.08 | 1.76 | 3.37 | 4.49 | 22.28 |
| WNW | 0.16 | 1.44 | 1.92 | 2.24 | 1.44 | 5.77 |
| NW | 0.16 | 0.80 | 0.80 | 0.80 | 0.96 | 2.08 |
| NNW | 0.32 | 0.16 | 0.64 | 0.64 | 0.00 | 0.00 |

CALM OBS = 61 PERCENT CALM = 9.78

Figure III.16: Wind rose diagram for St. John's, Month 16 (10.02.2003 - 07.03.2003).




PERCENT OCCURRENCE: Wind Speed ( Kilometers Per Hour)
LOWER BOUND OF CATEGORY

| DIR | 0 | 6 | 12 | 18 | 24 | 30 |
|---|---|---|---|---|---|---|
| N | 0.00 | 0.00 | 0.62 | 0.77 | 0.00 | 0.15 |
| NNE | 0.00 | 0.00 | 0.62 | 0.15 | 0.00 | 0.31 |
| NE | 0.31 | 0.31 | 0.31 | 0.62 | 0.00 | 0.00 |
| ENE | 0.62 | 0.62 | 0.00 | 0.15 | 0.00 | 0.15 |
| E | 1.23 | 0.77 | 0.31 | 0.93 | 0.00 | 0.00 |
| ESE | 0.77 | 0.77 | 0.62 | 0.15 | 0.00 | 0.00 |
| SE | 0.15 | 0.93 | 0.15 | 0.00 | 0.00 | 0.00 |
| SSE | 0.77 | 0.31 | 0.00 | 0.00 | 0.00 | 0.00 |

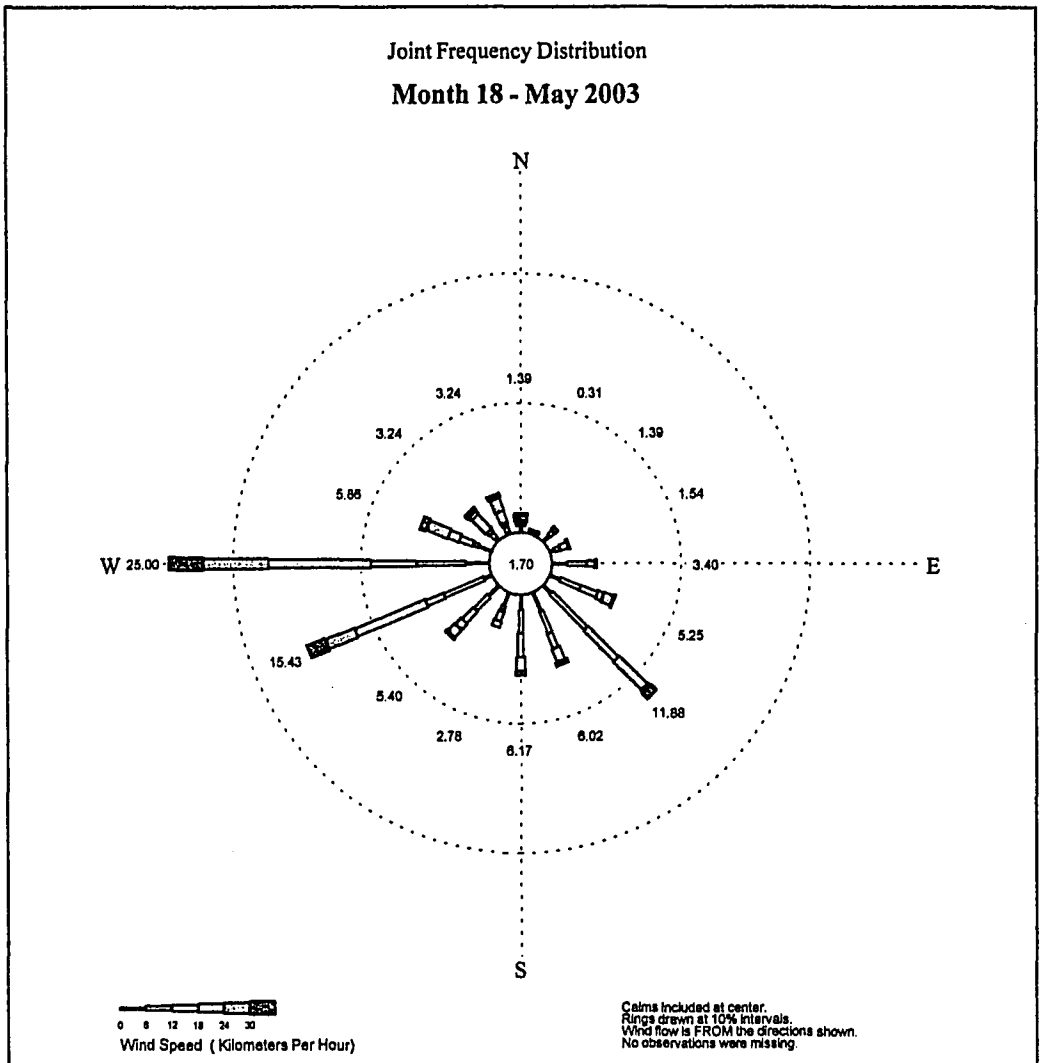
TOTAL OBS = 648 MISSING OBS = 0

PERCENT OCCURRENCE: Wind Speed ( Kilometers Per Hour)
LOWER BOUND OF CATEGORY

| DIR | 0 | 6 | 12 | 18 | 24 | 30 |
|---|---|---|---|---|---|---|
| S | 0.46 | 1.70 | 0.46 | 0.46 | 2.16 | 2.62 |
| SSW | 0.62 | 0.62 | 0.62 | 2.01 | 1.70 | 1.39 |
| SW | 0.15 | 0.93 | 2.93 | 2.01 | 1.08 | 2.01 |
| WSW | 0.77 | 2.62 | 4.48 | 6.17 | 3.40 | 2.31 |
| W | 1.54 | 3.24 | 2.62 | 4.17 | 3.24 | 5.86 |
| WNW | 0.31 | 0.93 | 0.93 | 1.85 | 2.47 | 3.09 |
| NW | 0.00 | 0.31 | 0.62 | 0.93 | 1.23 | 1.54 |
| NNW | 0.00 | 0.15 | 0.62 | 1.54 | 0.77 | 1.54 |

CALM OBS = 21 PERCENT CALM = 3.24

Figure III.17: Wind rose diagram for St. John's, Month 17 (09.03.2003 - 04.04.2003).




PERCENT OCCURRENCE: Wind Speed ( Kilometers Per Hour)
LOWER BOUND OF CATEGORY

| DIR | 0 | 6 | 12 | 18 | 24 | 30 |
|---|---|---|---|---|---|---|
| N | 0.46 | 0.00 | 0.00 | 0.15 | 0.15 | 0.62 |
| NNE | 0.15 | 0.00 | 0.00 | 0.00 | 0.15 | 0.00 |
| NE | 0.62 | 0.46 | 0.31 | 0.00 | 0.00 | 0.00 |
| ENE | 0.31 | 0.46 | 0.62 | 0.00 | 0.15 | 0.00 |
| E | 1.08 | 1.54 | 0.62 | 0.15 | 0.00 | 0.00 |
| ESE | 0.77 | 1.70 | 1.39 | 0.46 | 0.77 | 0.15 |
| SE | 0.77 | 3.86 | 3.40 | 3.09 | 0.15 | 0.62 |
| SSE | 1.54 | 1.85 | 1.23 | 1.08 | 0.00 | 0.31 |

TOTAL OBS = 648 MISSING OBS = 0

PERCENT OCCURRENCE: Wind Speed ( Kilometers Per Hour)
LOWER BOUND OF CATEGORY

| DIR | 0 | 6 | 12 | 18 | 24 | 30 |
|---|---|---|---|---|---|---|
| S | 1.08 | 1.85 | 1.85 | 1.08 | 0.31 | 0.00 |
| SSW | 1.08 | 0.46 | 0.77 | 0.46 | 0.00 | 0.00 |
| SW | 1.08 | 1.54 | 1.23 | 0.62 | 0.77 | 0.15 |
| WSW | 0.62 | 3.40 | 1.54 | 5.86 | 2.47 | 1.54 |
| W | 1.85 | 3.86 | 3.55 | 8.02 | 5.09 | 2.62 |
| WNW | 1.08 | 0.46 | 1.08 | 0.93 | 1.85 | 0.46 |
| NW | 0.31 | 0.46 | 0.31 | 1.54 | 0.31 | 0.31 |
| NNW | 0.15 | 0.46 | 0.31 | 0.93 | 1.08 | 0.31 |

CALM OBS = 11 PERCENT CALM = 1.70

Figure III.18: Wind rose diagram for St. John's, Month 18 (06.04.2003 - 02.05.2003).