Investigation of an introduced subtropical alga (*Lyngbya wollei*) in Whiteshell Provincial Park, Manitoba

by

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A Thesis
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In Partial Fulfillment of the Requirements
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Abstract

Nuisance growth of a filamentous cyanobacterium, *Lyngbya wollei*, was studied in lakes of Whiteshell Provincial Park in eastern Manitoba. Its increasing abundance in two lakes, White and Betula, over the last half dozen years has heightened awareness and concern by cottagers and recreational users that nearby lakes may become infested. *L. wollei* is typically found in the southeastern United States, and this is the only known occurrence in Canada. Desiccation experiments used to assess the ability of *L. wollei* to survive inter-lake transfer on recreational watercraft showed it can remain viable after short-term drying and stagnant conditions. Comparisons of lake basin morphology, land use, water and sediment chemistry of the infested lakes to those of other Whiteshell lakes showed that all lakes with shallow littoral areas and a photic depth greater than two and a half meters possess potential *L. wollei* habitat. Intensively used lakes such as Falcon, Caddy, Brereton, and Big Whiteshell are most susceptible to *L. wollei* inoculation. Principle Component Analysis based on water and sediment chemistry identified Jessica, Red Rock, Florence, and Madge Lakes as having the most similar conditions to the infested lakes. Consequently, these lakes may develop the greatest biomass and adverse effects of *L. wollei* growth. It is imperative that all clothing, footwear and recreational watercraft be cleaned thoroughly after being removed from infested lakes to prevent the further spread of *L. wollei* in the lakes of Whiteshell Provincial Park.

Chapter 1: Introduction

As humans become more mobile, moving efficiently around the globe, so do flora and fauna. Exotic species invade habitats where they do not naturally occur and often decrease both the abundance and diversity of native species in the invaded community (Buchan and Padilla 2000). This alters the system's natural biodiversity and community structure. Currently, exotic species constitute 10-30 % of the flora in most regions and, once established, are often impossible to eradicate (Buchan and Padilla 2000). As a result, inoculation must be minimized through the identification and protection of areas vulnerable to invasion.

L. wollei is a large, filamentous, mat forming cyanobacterium that has become common in lentic ecosystems in the Southeastern United States. At the time of this writing Lake Itasca in Minnesota is the most northerly known location of *L. wollei* in the United States. In Canada, *L. wollei* has been identified in two Whiteshell lakes, Betula and White. Presently, these infestations represent the only known occurrence of *L. wollei* in Canada. Recent *L. wollei* growth observed in the infested lakes and its occurrence around developed areas with cottages, resorts, campgrounds and public boat launches, suggest this is an exotic species introduced to the Whiteshell.

Like other exotic species, *L. wollei* is an opportunistic species out-competing most interspecific competitors once established in an aquatic system. Thick, dense mats proliferate on the bottom of littoral areas impeding swimming, boating and other recreational activities. Additionally the production of geosmin and neurotoxins analogous to those which cause paralytic shellfish poisoning may pose a health concern for all users (Carmichael *et al.* 1997). As a result, *L. wollei* infestations result in recreational, ecological and economic loss of affected lakes (Speziale *et al.* 1991).

Whiteshell Provincial Park encompasses 131 lakes (Schneider 2002). The Whiteshell has historically been an important recreational area facilitating activities such as swimming, boating and fishing. Betula and White Lakes have active water skiing clubs which were established in 1950 and 1963 respectively. These are the only two Whiteshell lakes with ski clubs. Historically boats used in water skiing competitions were moved around the continent for competitions. If *L. wollei* is transported between lakes by recreational watercrafts it is imperative that other Whiteshell lakes are protected from *L. wollei* inoculation.

This thesis marks the beginning of the investigation, identification and monitoring of *L. wollei* infestations in Whiteshell Provincial Park. The research presented here may be used to aid future research endeavors so that Whiteshell lakes may continue to provide recreational opportunities for future generations.

1.1 Objectives

1) The primary objective of this study was to examine the distribution and inoculation source of *L. wollei* in Whiteshell Provincial Park.

2) The second objective of this study was to investigate why Betula and White Lakes are the only known Whiteshell lakes with *L. wollei* infestations. The infested lakes were compared to other Whiteshell lakes based on land-use parameters, lake morphology, water and sediment chemistry.

1.2 Hypotheses

1) I hypothesize that recreational watercraft moved between infested water bodies in the United States and the Whiteshell resulted in the infestation of Betula and White Lakes. Current boat movement between these infested lakes and other Whiteshell lakes may put other waterbodies in the Whiteshell and throughout North America at high risk for infestation.

2) I hypothesize that Betula and White Lakes are unique from other Whiteshell lakes, with certain parameters which enable *L. wollei* to proliferate. Unique parameters may include high concentrations of calcium and phosphorus, which are known to be growth limiting nutrients. If Betula and White Lakes do not prove to be unique from other Whiteshell lakes based on land-use, lake morphology, water or sediment chemistry then we may use the data to predict other similar Whiteshell lakes which may be susceptible to *L. wollei* inoculation and infestation.

Chapter 2: Literature Review

2.1 Lyngbya wollei

2.11 Introduction

In the southeastern United States, a nuisance mat-forming cyanobacterium impedes the recreational, economic and aesthetic value of infested aquatic systems (Speziale *et al.* 1991). Recent taxonomic investigation by Speziale and Dyck (1992) classified the nuisance species as *Lyngbya wollei* Farlow ex Gomont comb. nov. Classification has enabled consistent communication and biological understanding of *L. wollei.* Investigation of its ecological and biochemical characteristic has resulted in a better understanding of this opportunistic species. *L. wollei* possesses both morphological and physiological characteristics, that give it a competitive advantage over other species. Consequently, aquatic systems can become dominated by *L. wollei* with associated biomass reaching as high as 1.0 – 1.5 kg dry weight m^{-2} (Beer *et al.* 1986). The nuisance potential of *L. wollei* makes it crucial to focus on management techniques so that this species no longer limits the use of the reservoirs, lakes and ponds which it currently infests.

2.12 Taxonomy

In the last three decades infestations of a recently described and exceptionally large filamentous cyanobacterium have become increasingly common in the Southeastern United States. Although there have been consistent descriptions of this organism it has been identified as six different species within three genera (Table 1) (Speziale and Dyck 1992). Proper identification of this nuisance organism is crucial for consistent communication and biological understanding. Traditionally cyanobacteria have been taxonomically classified by the International Code of Botanical Nomenclature ($ICBN_1$). This system is primarily used for eukaryotic organisms. Some researchers believe that cyanobacteria, with their prokaryotic habit, would be more accurately classified by the rules of the International Code of Bacteriological Nomenclature ($ICBN_2$) (Rippka *et al.* 1979). The discrepancies in the

Table 1: Inaccurate taxonomic classifications of *Lyngbya wollei* Farlow *ex* Gomont (Speziale and Dyck 1992).

Proposed Taxonomic Classification	Author(s) cited
Microcoleus lyngyaceus	(Kutzing) Crouan *sensu* Drouet
Plectonema wollei	Farlow *et* Gomont
Lyngbya magnifica	Gardner
Lyngbya majuscula	Harvey *ex* Gomont
Lyngbya birgei	Smith
Lyngbya latissima	Prescott

identification of this cyanobacterium appear to be the result of concurrent use of the two classification systems by different groups of researchers. Use of the $ICBN_2$ with cyanobacteria is based on a limited number of strains, and therefore receives limited support (Speziale and Dyck 1992).

The use of the $ICBN_1$ is also problematic. Grietler sub-divided the family Homogoneae into two sub-families using this system. Sub-division was based on the occurrence of heterocysts and presence of false branching (Speziale and Dyck 1992). The family Oscillatoriaceae is composed of taxa, that lack heterocysts and have simple filaments. *Lyngbya,* although occasionally having false branches, was placed in this family. *Plectonema* was placed in the sub-family Scytonemataceae due to characteristic false branching, even though it lacked heterocysts. Since both genera lack heterocysts the division was ultimately based on the occurrence of false branching. Speziale and Dyck (1992) proposed that false branching is a taxonomically invalid method of classification because it can result from environmental modification.

In contrast to *Plectonema, Lyngbya* does not put undo emphasis on the environmentally variable false branching characteristic. Therefore Speziale and Dyck (1992) has proposed that *Lyngbya* is the most accurate genus classification of the nuisance organism. Designation of the specific epithet was based on the elimination of previously applied taxonomic classifications. *Lyngbya birgei* is a planktonic species, which does not form floating masses. The nuisance organism differs from the marine species *Lyngbya majuscula* both in size and it has a limited halotolerance. *Lyngbya latissima* and *Lyngbya magnifica* are acknowledged synonyms for *Plectonema wollei. Plectonema wollei* is accurate in the description of the nuisance organism yet it puts strong emphasis on false branching. As a result, the nuisance organism has been classified as *Lyngbya wollei* Farlow ex Gomont comb. nov (Speziale and Dyck 1992).

2.13 Morphology

Speziale and Dyck (1992) identified a specimen type based on morphological variation found in *L. wollei* specimens. *L. wollei* cells are defined as discoid in shape having a diameter of 24-65mðm. Cells vary in length from 2-12 mm. The cells are arranged into uniseriate filaments, which are encased by a hyaline, lamellate sheath up to 12 mðm thick. The filaments are indeterminate in length and can exceed 40 cm in length. False branching may result from protrusion through lateral breaks in the sheath. These filaments produce an entangled mat of sparsely branched filaments. Mats are vertically stratified occurring throughout the water column in the summer (Speziale *et al.* 1991). The subsurface filaments are photosynthetically active, rich in phycobilin and as a result are blue black in color. Surface filaments are photosynthetically inactive and they are yellow – orange in colour, as a result of the high carotenoid content and bleached chlorophyll and phycobilin (Speziale and Dyck 1992). *L. wollei* is a perennial species, which overwinters as a benthic mat. *L. wollei* has no specialized reproductive or overwintering structures such as akinetes. All biomass produced throughout the water column accumulates as a benthic mat which functions as a base stock for re-infestation in the following growth season. Heterotrophic *L. wollei* filaments buried in the benthos are living and viable (Speziale *et al.* 1991). As a result these filaments may function as inoculum for subsequent growth (Head *et al.* 1999).

2.14 Ecological Adaptation

In any ecological system species assemblage is determined by the chemical and physical characteristics of the environment (Kohler and Hoeg 2000). In *L. wollei* dominated habitats these characteristics have been investigated to identify optimal growth conditions. Water conductivity and alkalinity were found to account for 55% of the variability in *L. wollei* biomass (Cowell and Botts 1994). Limited halotolerance suggests that *L. wollei* is a freshwater species. *L. wollei* is a stenohaline species with a strong preference for freshwater, showing negative growth at all salinities between 0 and 35 ppt by the loss of cells. At salinities of 17.5ppt and above, rapid death of the organism occurs (Cowell and Botts 1994). Optimal growth of *L. wollei* occurs in water of pH 8 (Cowell and Botts 1994). It may be concluded that *L. wollei* is sensitive to acidity and grows optimally in an alkaline environment. In contrast, at pH 4 there was negligible growth (Tubea *et al.* 1981). *L. wollei* has been identified most commonly in the southwestern United States in freshwater lentic ecosystems with these characteristics. Lakes, ponds and reservoirs may all be affected.

Alkalinity reflects the predominant form of inorganic carbon present in the water body and thus is an essential component in understanding the niche of *L. wollei.* Photosynthetic activity of *L. wollei* results in diel fluctuations in dissolved inorganic carbon (DIC) (Beer *et al.* 1990). In initial hours of photosynthesis, CO_2 concentrations are depleted to negligible levels. The water pH increases and consequently increases the bicarbonate concentration (Beer *et al.* 1986). At pH 8 bicarbonate is the predominant form of DIC. Beer *et al.* (1992) report that CO_2 concentrations within *L. wollei* mats are less than 1mðM suggesting bicarbonate is the main source of DIC available to *L. wollei* for photosynthesis and growth. Bicarbonate concentrations are as low as 0.15mM at midday as a result of increasing pH and yet photosynthesis still functions at 60% of its maximum productivity (Beer *et al.* 1986). Beer *et al.* (1986) results conclude efficient inorganic carbon uptake at low DIC levels maintains photosynthetic productivity.

L. wollei is able to efficiently utilize bicarbonate to supply cellular demands for inorganic carbon. This provides a competitive advantage to the species (Beer *et al.* 1986). Bicarbonate use results in an increase in carbon dioxide levels at the ribulose – 1,5-bisphosphate carboxylase oxygenase (RUBISCO) site. This is the same result achieved by plants employing the C4 mechanism (Beer *et al.* 1990). Beer *et al* (1992) conclude that there is no evidence supporting bicarbonate dehydration at the cell wall. Instead, *L. wollei* achieves saturating inorganic carbon concentrations through cellular uptake of bicarbonate. This conclusion is based on the absence of the enzyme carbonic anhydrase (CA) which intraconverts bicarbonate and carbon dioxide. In addition there is no evidence of a highly active carbon dioxide transport system and oxygen released originates internally. It is further