



WATER RESOURCES RESEARCH GRANT PROPOSAL

1. **TITLE** : " A SURVEY of 50 NH LAKES for MICROCYSTINS".

2. **FOCUS CATEGORIES** : TS, WQL, ECL

3. **KEYWORDS** : *Microcystis*, secondary metabolites, biotoxins, microcystins, cyanobacteria, algae, ecosystems, lakes, nutrients, phosphorous/nitrogen, toxic substances, water chemistry, water quality monitoring.

4. **DURATION** : September 1, 1998 - November 30, 2000.

5. **FEDERAL FUNDS REQUESTED** : \$ 73,688 (2 years)

6. **NON-FEDERAL MATCHING FUNDS** : \$ 75,505 (2 years)

7. **PRINCIPAL INVESTIGATORS** : James F. Haney & Miyoshi Ikawa

Dept. of Zoology, University of New Hampshire.

8. **CONGRESSIONAL DISTRICT** : FIRST

9. **Statement of regional or state water problem** :

Naturally-occurring biotoxins in aquatic food chains have received considerable attention recently in the scientific literature and the popular media. The formation of a center for the study of Harmful Algal Blooms (HABs) by the International Oceanographic Commission, the Water resources guide to managing toxic cyanobacterial blooms (Yoo, *et al.*, 1995) and the EPA-sponsored "Status Report on Planktonic Cyanobacteria and their Toxins" by Carmichael (1992) all point to the need for accurately assessing biotoxins from microorganisms commonly found in aquatic environments. Over the last 4 summers, we have measured microcystin levels in Silver Lake, Hollis, NH and found significant microcystin concentrations in *Microcystis* cells (2500 µg/g dry wt), zooplankton (60 µg/g dry wt), benthic bivalves (7 µg/g dry wt.) and dissolved in lakewater filtrates (0.4 - 20 µg/L). The latter result is noteworthy since it exceeds the guideline of 1 µg/L suggested for safe exposure for recreation and drinking (Steffensen and Nicholson, 1994).

Most field studies of cyanobacteria toxins have examined eutrophic lakes with conspicuous surface blooms of algae. Thus it is often assumed that the problem of biotoxins in lakes is confined to such problem lakes and linked directly to the problem of eutrophication. There are several lines of evidence that suggest this assumption should be tested. First, until recently, the HPLC method of analysis has limited the detection of microcystins to lakes with high concentrations of phytoplankton. Using freshwater mussels as biomagnifiers, Prepas, *et al.* (1997) demonstrated the presence of microcystins in oligotrophic lakes. Secondly, not all cyanobacteria species require high nutrient levels. For example, *Microcystis aeruginosa*, an important producer of microcystins, is present in most of the lakes in New Hampshire, and is the dominant phytoplankter in the meso-eutrophic Silver Lake as well as in Russell Pond, an ultraoligotrophic lake. In a recent preliminary survey, we have also detected significant quantities of microcystins in 9 other NH lakes with varied trophic conditions (Figure 1).

10. Statement of results or benefits :

Toxic cyanobacteria blooms have been reported from at least 32 countries, including 16 in Europe, 5 Canadian Provinces and 27 states in the US (Carmichael, 1992). In the 1980's, toxic cyanobacterial blooms in New Hampshire were reported, intermittently, in at least 30 lakes, ponds and reservoirs, some with environmental, legal, and health-related implications. On at least two occasions, NH Public Health officials reported approximately 100 people with gastroenteritis and flu-like symptoms that occurred after contact with lake water at NH State Parks in which *Microcystis* blooms dominated the phytoplankton. Eutrophication has been linked to cyanobacterial blooms with increasing frequency in freshwater lakes, ponds and drinking water supplies. The blooms are often, but not always, accompanied by toxins or metabolites which can cause mortality of vertebrate animals and impact various planktonic, benthic and pelagic components of aquatic communities. Planktonic cyanobacteria are often sources of several types of neurotoxins and of a family of hepatotoxins, the latter liver toxins causing the most problems on a worldwide scale. These hepatotoxins, called microcystins, are produced by several cyanobacteria taxa including *Microcystis*, *Anabaena*, *Nodularia*, *Oscillatoria*, all represented in the plankton of various lakes in NH. Microcystins are potent offenders and comprise a chemically-related family of slow acting, cyclic, heptapeptides, which produce internal bleeding in liver tissues of vertebrate animals. The reported LD50 for microcystin in mammals is 50 µg/Kg body weight. Microcystins alter intracellular microfilament structure (Yoshizawa, *et al.* 1990), inhibit protein phosphatase activity (PP1 and PP2a) (Honkanen, *et al.* 1991) and promote tumor growth (Falconer, 1991). They have been implicated in the poisoning of humans, cattle, sheep, waterfowl, dogs and salmonid fishes (Galey, *et al.* 1987; Watanabe, *et al.* 1996). The impact of human exposure to *Microcystis* is noted in health reports from water supplies in Australia, Brazil, Canada and from Harare, Zimbabwe, where blooms have been linked to seasonal outbreaks of gastroenteritis in children (Zillberg, 1996). Microcystins have recently been related to the deaths of reared Atlantic salmon, the anomaly called net-pen liver disease (NLD), in British Columbia, Canada and Washington State (Anderson, *et al.* 1993) and the inhibition of osmoregulatory mechanisms in freshwater fishes (Gaete, *et al.* 1994).

A primary result of this study will be the assessment of the presence and amount of microcystin biotoxins in 50 NH lakes of varied trophic status. Empirical regression models will be developed to predict microcystin concentrations based on the indicators of eutrophication, such as total phosphorous concentration and chlorophyll-a. Cluster analysis will be used to identify lake associations, grouped according to levels of microcystins, as well as physical, chemical and biological features of the lakes. We will also identify the compartments in the lake that contain the microcystins, i.e. lakewater (dissolved), phytoplankton and zooplankton. Comparisons with the composition of the phytoplankton will indicate which species of cyanobacteria produce the toxins. The amount of microcystins in the zooplankton will show the degree of bioaccumulation of the toxin by the algal grazers and indicate the potential for bioaccumulation and biomagnification of toxins further up the food chain, such as in fish. Based on our results from the 50 lakes in the study, we will attempt to develop a ranking of other lakes in the state according to their "biotxin sensitivity", an index of the likelihood of the occurrence of microcystin in a given lake.