WATER RESOURCES RESEARCH GRANT PROPOSAL

1. Title: Stable Isotope Analysis of the Contribution of N_2 Fixation to Phytoplankton Nutrition, Lake Nitrogen Budgets and Lake Eutrophication

2. Focus categories: NU, WQ, SW

3. Keywords: Algae, eutrophication, foodwebs, geochemistry, lakes, nitrogen, nitrogen fixation, nonpoint source pollution, nutrients, nutrient limitation, stable isotopes, water quality

4. Duration: 8/1/98 - 7/31/00

5. Fiscal year 1998 Federal funds:	56,512	56,512	0
	Total	Direct	Indirect
6. Non-Federal funds allocated:	113,026	111,538	169,538
	Total	Direct	Indirect

7. Principal Investigators: Suzanne N. Levine and Andrea Lini, University of Vermont, Burlington, VT.

8. Congressional District: 1st District, State of Vermont

9. Statement of critical regional or state water problems:

Of all the water quality problems faced by the United States during the twentieth century, eutrophication has been the most pervasive, seriously affecting hundreds of thousands of lakes and streams. Management of eutrophication has focused on controlling phosphorus levels in municipal and industrial discharges and in non-point source runoff, but little headway has been made. The incidence of eutrophication among U.S. lakes and streams is just as great today as 30 years ago (U.S. EPA 1994). Blame has been placed on population growth, inadequate funding of treatment plant construction and ineffective non-point-source programs. However, another factor may explain part of the low success rate. A recent review of studies of nutrient limitation in North American lakes (Elser et al. 1990) indicates that nitrogen limitation of algal growth may be more common than is generally believed. While nutrient limitation assays undertaken by the U.S. EPA during the 1970's suggested that roughly 72% of U.S. lakes were P limited, while only 16% were N limited, the new data suggest nearly equal incidence of P and N limitation (47 versus 40%; Elser et al. 1990). The two data sets differ in their conclusions because the former was based on assays with a test alga grown under laboratory conditions, and whose P requirements appear to be exceptionally high (Rhee and Gotham 1980), while the latter was based on assays performed *in situ* with natural phytoplankton communities. If N limitation is as common in lakes as the new survey suggests, then current concepts about biomass regulation in lakes must be revised, and management strategies for minimizing eutrophication reassessed. The project proposed here would reexamine one concept basic to current eutrophication theory: the idea that N limitation is scarce in lakes due to the existence of cyanobacteria (blue green algae) with the ability to fix atmospheric N (Schindler 1977). Because the atmospheric reservoir of N_2 is vast, the ability of these algae to draw N into a lake is viewed as essentially unlimited. The N fixation hypothesis has had a major influence on lake and watershed management. While frequent N limitation in lakes would suggest improved water quality as a result of N reductions in runoff, P limitation due to N fixation argues against N control. Reductions in N levels have been viewed as futile, as the removed N can be regained through fixation, and even counterproductive, because the algae responsible for fixation are largely the same species that form nuisance surface blooms (e.g., *Aphanizomenon* and *Anabaena*; one bloom former, *Microcystis*, does not fix N).

While the N fixation hypothesis has been widely quoted, it has not been thoroughly tested. This is largely because the tasks involved are difficult, and funding for in-lake studies meager. N fixation is a light dependent process (Lewis and Levine 1984). Therefore, changing light intensities and the relationship between fixation rates and light must be assessed to estimate whole lake fixation rates. Furthermore, the fixers migrate vertically over the course of a day and are vacuolated so that they are subject to wind rafting (Levine and Lewis 1984, 1985). Many samples must be taken just to evaluate the spatial distribution of the algae. To determine phytoplankton demand for N and N acquisition through dissolved inorganic N uptake, additional assays have been necessary. The limited data available (about a dozen lakes have been analyzed) indicate that N fixation can be substantial; in some lakes >50% of the N supply is atmospheric (see the reviews by Dierberg and Scheinkman 1987; Howarth et al. 1988). However, even high N fixation rates do not appear to ensure P-limited growth. Both Leonardson (1984) and Levine and Lewis (1987) describe lakes with high N fixation rates but multiple symptoms of N limitation. In these lakes, N fixation appears to be constrained by the mixing of phytoplankton into regions of low light intensity, or by other environmental factors. Clearly, more studies are needed before generalizations can be made about the role of N fixation in the eutrophication process. The project proposed here would roughly double the information currently available on N fixation as a source of N for phytoplankton and as an input to lake ecosystems, while providing a new method that we believe will encourage additional research. Our basic approach will be natural abundance stable isotope analysis. Stable isotope methodology is much simpler than process measurement and mass balance, and probably more accurate as well. It requires no manipulation of phytoplankton communities or of nutrient concentrations, no incubations, and no tracking of light intensities or algal distribution. Because stable isotopes integrate spatially and over several-day periods, a single sampling of water and phytoplankton will provide as much information as conventional multi-site, multi-day analyses. We also plan to measure a suite of environmenatal variables (light, temperature, pH, and N, P, and C availability) to evaluate controls on fixation rates, and to conduct nutrient limitation assays to assess the effectiveness of N fixation in alleviating N limitation.

10. Statement of results or benefits:

Anticipated products of the project are:

1. A tested method for estimating the relative importance of dissolved inorganic nitrogen (DIN) and atmospheric nitrogen as sources of N for algae, and application of the method to 15 Vermont lakes;

2. A first time evaluation of the relative importance of N and P as limiting nutrients in Vermont lakes;

3. Information on the environmental factors influencing the rise to dominance of N fixers and the initiation of N fixation;

4. A tested method for estimating the importance of N-fixing cyanobacteria as food for zooplankton, and its application to 3 Vermont lakes; and

5. An evaluation of the amount of fixed N in the surface sediments of 15 Vermont lakes. This approach may prove useful in indicating average lake trophic status over periods of 10-50 years.

In short, we believe that the outcome of this project could influence concepts about nutrient limitation, about how N enters lakes and is cycled, and about controls on cyanobacterial biomass. From a management perspective, our study will provide guidance on the issue of whether managers should control N levels in runoff to improve lake water quality. Our findings will be relevant nationally and globally, as well as within the northeastern U.S.