



**Lake Champlain
Basin Program**

Lower Trophic Level Interactions In The Pelagic Foodweb Of Lake Champlain

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Lake Champlain Basin Program Technical Reports

1. *A Research and Monitoring Agenda for Lake Champlain.* Proceedings of a Workshop, December 17-19, 1991, Burlington, VT. Lake Champlain Research Consortium. May, 1992.
2. *Design and Initial Implementation of a Comprehensive Agricultural Monitoring and Evaluation Network for the Lake Champlain Basin.* NY-VT Strategic Core Group. February, 1993.
3. (A) *GIS Management Plan for the Lake Champlain Basin Program.* Vermont Center for Geographic Information, Inc., and Associates in Rural Development. March, 1993.

(B) *Handbook of GIS Standards and Procedures for the Lake Champlain Basin Program.* Vermont Center for Geographic Information, Inc. March, 1993.

(C) *GIS Data Inventory for the Lake Champlain Basin Program.* Vermont Center for Geographic Information, Inc. March, 1993.
4. (A) *Lake Champlain Economic Database Project. Executive Summary.* Holmes & Associates. March 1993.

(B) *Socio-Economic Profile, Database, and Description of the Tourism Economy for the Lake Champlain Basin.* Holmes & Associates. March 1993

B) *Socio-Economic Profile, Database, and Description of the Tourism Economy for the Lake Champlain Basin. Appendices.* Holmes & Associates. March 1993

(C) *Potential Applications of Economic Instruments for Environmental Protection in the Lake Champlain Basin.* Anthony Artuso. March 1993.

(D) *Conceptual Framework for Evaluation of Pollution Control Strategies and Water Quality Standards for Lake Champlain.* Anthony Artuso. March 1993.
5. *Lake Champlain Sediment Toxics Assessment Program. An Assessment of Sediment - Associated Contaminants in Lake Champlain - Phase 1.* Alan McIntosh, Editor, UVM School of Natural Resources. February 1994.

Lake Champlain Sediment Toxics Assessment Program. An Assessment of Sediment - Associated Contaminants in Lake Champlain - Phase 1. Executive Summary. Alan McIntosh, Editor, UVM School of Natural Resources. February 1994.
6. (A) *Lake Champlain Nonpoint Source Pollution Assessment.* Lenore Budd, Associates in Rural Development Inc. and Donald Meals, UVM School of Natural Resources. February 1994.

(B) *Lake Champlain Nonpoint Source Pollution Assessment. Appendices A-J.* Lenore Budd, Associates in Rural Development Inc. and Donald Meals, UVM School of Natural Resources. February 1994.

7. *Internal Phosphorus Loading Studies of St. Albans Bay. Executive Summary.* VT Dept of Environmental Conservation. March 1994.
 - (A) *Dynamic Mass Balance Model of Internal Phosphorus Loading in St. Albans Bay, Lake Champlain.* Eric Smeltzer, Neil Kamman, Karen Hyde and John C. Drake. March 1994.
 - (B) *History of Phosphorus Loading to St. Albans Bay, 1850 - 1990.* Karen Hyde, Neil Kamman and Eric Smeltzer. March 1994.
 - (C) *Assessment of Sediment Phosphorus Distribution and Long-Term Recycling in St. Albans Bay, Lake Champlain.* Scott Martin, Youngstown State University. March 1994.
8. *Lake Champlain Wetlands Acquisition Study.* Jon Binhammer, VT Nature Conservancy. June 1994.
9. *A Study of the Feasibility of Restoring Lake Sturgeon to Lake Champlain.* Deborah A. Moreau and Donna L. Parrish, VT Cooperative Fish & Wildlife Research Unit, University of Vermont. June 1994.
10. *Population Biology and Management of Lake Champlain Walleye.* Kathleen L. Newbrough, Donna L. Parrish, and Matthew G. Mitro, Fish & Wildlife Research Unit, University of Vermont. June 1994.
11. (A) *Report on Institutional Arrangements for Watershed Management of the Lake Champlain Basin. Executive Summary.* Yellow Wood Associates, Inc. January 1995.
 - (B) *Report on Institutional Arrangements for Watershed Management of the Lake Champlain Basin.* Yellow Wood Associates, Inc. January 1995.
 - (C) *Report on Institutional Arrangements for Watershed Management of the Lake Champlain Basin. Appendices.* Yellow Wood Associates, Inc. January 1995.
12. (A) *Preliminary Economic Analysis of the Draft Plan for the Lake Champlain Basin Program. Executive Summary.* Holmes & Associates and Anthony Artuso. March 1995
 - (B) *Preliminary Economic Analysis of the Draft Plan for the Lake Champlain Basin Program.* Holmes & Associates and Anthony Artuso. March 1995
13. *Patterns of Harvest and Consumption of Lake Champlain Fish and Angler Awareness of Health Advisories.* Nancy A. Connelly and Barbara A. Knuth. September 1995.
14. (A) *Preliminary Economic Analysis of the Draft Plan for the Lake Champlain Basin Program. Executive Summary - Part 2.* Holmes & Associates and Anthony Artuso. November 1995
 - (B) *Preliminary Economic Analysis of the Draft Plan for the Lake Champlain Basin Program - Part 2.* Holmes & Associates and Anthony Artuso. November 1995
15. *Zebra Mussels and Their Impact on Historic Shipwrecks.* Lake Champlain Maritime Museum. January 1996.

16. *Background Technical Information for Opportunities for Action: An Evolving Plan for the Future of the Lake Champlain Basin.* Lake Champlain Basin Program. June 1996
17. (A) *Executive Summary. Economic Analysis of the Draft Final Plan for the Lake Champlain Management Conference.* Holmes & Associates and Anthony Artuso. July 1996

(B) *Economic Analysis of the Draft Final Plan for the Lake Champlain Basin Management Conference.* Holmes & Associates and Anthony Artuso. July 1996
18. *Catalog of Digital Spatial Data for the Lake Champlain Basin .* Vermont Center for Geographic Information, Inc. September 1996.
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20. *Understanding Phosphorus Cycling, Transport and Storage in Stream Ecosystems as a Basis for Phosphorus Management.* Dr. James P. Hoffmann, Dr. E. Alan Cassell, Dr. John C. Drake, Dr. Suzanne Levine, Mr. Donald W. Meals, Jr., Dr. Deane Wang. December 1996.
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22. *Characterization of On-Farm Phosphorus Budgets and Management in the Lake Champlain Basin.* Robert D. Allshouse, Everett D. Thomas, Charles J. Sniffen, Kristina Grimes, Carl Majewski - Miner Agricultural Research Institute. April 1997
23. (A) *Lake Champlain Sediment Toxics Assessment Program. An Assessment of Sediment - Associated Contaminants in Lake Champlain - Phase 11. Executive Summary.* Alan McIntosh, Mary Watzin and Erik Brown, UVM School of Natural Resources. October 1997

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24. *Development of Land Cover/Land Use Geographic Information System Data Layer for the Lake Champlain Basin and Vermont Northern Forest Lands Project Areas.* Dr. Thomas Millette. October 1997
25. *Urban Nonpoint Pollution Source Assessment of the Greater Burlington Area.* Urban Stormwater Characterization Project. James Pease, VT Dept. of Environmental Conservation. December 1997
26. *Long-Term Water Quality and Biological Monitoring project for Lake Champlain.* Cumulative Report for Project Years 1992- 1996.
27. *Cumberland Bay PCB Study.* Clifford W Callinan, NY State Dept. of Environmental Conservation; Lyn McIlroy, Ph.D., SUNY Plattsburgh; and Robert D. Fuller, Ph.D., SUNY Plattsburgh. October 1998.
28. *Lake Champlain Underwater Cultural Resources Survey. Volume 1: Lake Survey Background and 1996 Results.* Scott A. McLaughlin and Anne W. Lessman, under the direction of Arthur B. Cohn, Lake Champlain Maritime Museum. December 1998.

29. *Evaluation of Soil Factors Controlling Phosphorus Concentration in Runoff from Agricultural Soils in the Lake Champlain Basin.* Frederick R. Magdoff, William E. Jokela, and Robert P. Durieux, UVM Department of Plant and Soil Sciences. June 1997.
30. *Lower Trophic Level Interactions in the Pelagic Foodweb of Lake Champlain.* Dr. Suzanne N. Levine, Dr. Mark Borchardt, Dr. Moshe Braner, Angela Shambaugh, and Susan Spencer of UVM School of Natural Resources and Marshfield Medical Research Foundation. July 1997.

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LOWER TROPHIC LEVEL INTERACTIONS
IN THE PELAGIC FOODWEB OF LAKE CHAMPLAIN

FINAL REPORT
(Project LC-RC92-6-NYRFP)

submitted to
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TABLE OF CONTENTS

	Page
SECTION I: Executive Summary.....	1-1
SECTION II: General Introduction.....	2-1
SECTION III: Phosphorus, Nitrogen and Silica as Controls on Phytoplankton Biomass and Species Composition in Lake Champlain (USA-Canada).....	3-1
SECTION IV: The Impact of Zooplankton Grazing on Phytoplankton Species Composition and Biomass in Lake Champlain (USA-Canada)	4-1
SECTION V: Importance of Grazers in Controlling Bacterioplankton and Heterotrophic Protozoa in Lake Champlain.	5-1
SECTION VI: Within-Season Foodweb Model.....	6-1
SECTION VII: Final Discussion.....	7-1

SECTION I

EXECUTIVE SUMMARY

During 1994 and 1995, experiments were conducted on Lake Champlain for the purpose of identifying and quantifying trophic interactions within the microbial portion of the lake's foodweb. The study was funded by the U.S. Environmental Protection Agency and managed by the New England Interstate Water Pollution Commission. The investigative team consisted of three scientists from the School of Natural Resources at the University of Vermont, Dr. Suzanne Levine, Dr. Moshe Braner, and Ms. Angela Shambaugh, and two scientists from the Environmental Health Unit of the Marshfield Medical Foundation (WI), Dr. Mark Borchardt and Ms. Sue Spencer. Mr. Scott Quinn of the New York State Department of the Environment was the project officer. This report describes the objectives, methods and results of the research conducted. It also includes description of a numerical model developed as an aid to investigating possible implication of foodweb perturbations and to understanding natural seasonal trends in plankton communities.

Project Purpose and Design

Lake Champlain is highly valued by the residents of Vermont and New York for the recreational opportunities it provides, as well as its historical importance, immense size and beauty. In addition, the lake is of practical importance: it provides water for hundreds of thousands of people and is one of the main attractions of the Valley's \$2 billion per year tourist industry. While generally considered healthy, the lake is far from pristine. Excess nutrient loading, principally from municipal sewage and agriculture, has caused algal blooms and nuisance weed beds in some bays and shallow regions of the lake; toxic pollutants are present at unsafe levels in sediments near urban areas; and there have been multiple invasions of exotic biological species (most significantly, lamprey, milfoil, water chestnut and zebra mussel) with consequences for native communities that are largely unknown.

Management of Lake Champlain has focused on two goals: support of its economically important sports fishery through fish stocking and lamprey control, and alleviation of weed and algal problems through phosphorus reductions in runoff waters and municipal sewage outfall. The latter policy is based on the premise that algal assays performed by the EPA and local collaborators in the 1970's correctly identified phosphorus as the nutrient limiting growth in the lake, and that phosphorus continues to be the sole growth limiting factor twenty-five years after these analyses.

Lately, there has been concern that the two management goals, phosphorus reduction and fisheries improvements, may have contradictory elements. While a lake relatively free of algae and plants may look better, it provides less food for herbivorous invertebrates (zooplankton and zoobenthos). Invertebrates provide food for small forage and planktivorous fish, and these, in turn, feed the larger piscivores that are the basis of the sports fishery. A productive sports fishery therefore may require a fairly vigorous level of primary productivity.

It is also conceivable that fish stocking and lamprey control will influence algal standing stocks, although the mechanism of this outcome is more obscure and less predictable than foodweb alterations from the bottom up. It involves a phenomenon referred to as a "trophic cascade", in which upper trophic levels affect those below them through predator-prey interactions. As the "cascade" moves down from one trophic level to another, it exerts alternating positive and negative effects on population size. In the case of heavy fish stocking, the dense piscivore populations that are created are expected to feed heavily on the planktivorous fish that are their prey, causing planktivore densities to decline. Zooplankton populations once held in check by planktivore feeding then are released from this constraint and increase in density. In theory, the next step in the sequence of events, is a decline in algal biomass, as zooplankton feeding increases. This is, of course, the outcome managers would like, for it would result in improved water quality. Unfortunately, there are complexities within the foodweb which can yield a very different results. The existence of carnivorous zooplankton is one complication. These animals, which are fed upon by fish, also compete with the fish for

herbivores zooplankton prey. Thus they may act as an additional step, or partial step, in the cascade. Released from fish predation pressure, these animals may increase in numbers to make up for some of the lost fish cropping on herbivores. The final result would be less of a reduction in algal biomass through the cascade.

Yet another complicating factor is phytoplankton variability in their vulnerability to grazing. Some species escape grazing by being difficult to ingest, unpalatable, or toxic. Thus a community may "adapt" to increased grazing pressure with a species switch from edible to inedible species. The inedible species will largely escape predation and thus maintain a biomass similar to that present before the trophic cascade occurred (being limited in biomass by resource availability, which presumably doesn't change). Among the least edible of algae are the bluegreens that cause surface scums. Thus the final outcome of a trophic cascade might be degraded, rather than improved, algal conditions.

Finally, a trophic cascade may not complete its pathway from piscivores down to algae. If resources limit growth rates at any trophic level in the foodweb, reduced grazing pressure on that trophic level will fail to stimulate population increases (as there are no resources to support an expansion), and the trophic cascade will halt at that level.

What ecologists refer to as "ecological surprises" are common in lake management. These are situations in which lake management schemes yield unexpected results. The classic example is the collapse of salmon populations in several western U.S. lakes as a result of Mysis introduction. Mysis was added by enthusiastic managers as forage for sports fishes, but instead competed with the fishes for zooplankton food (Spencer et al. 1991). Most ecological surprises are caused by foodweb interactions that are not recognized as important when management plans are drawn up. Consequently, the best insurance against management disasters is familiarity with the structure and dynamics of foodwebs.

The Lake Champlain Management Conference recognized this maxim when they decided to fund three foodweb related projects on Lake Champlain: the biomonitoring program being carried out by the State of New York, the top predator bioenergetics project that was conducted

by another team of UVM scientists, and the "bottom up" project described in this report. Lake Champlain is a shamefully understudied lake for its size and its recreational and historical importance. While there have been occasional surveys of one biological group or another in the lake over the past century, neither large nor long-term commitments to research on the lake have been made. Monitoring of phytoplankton, zooplankton, and zoobenthos communities has been underway for the past five years, but it is in jeopardy of discontinuation due to insufficient funds. Heterotrophic protozoan and natural (noncoliform) bacterial populations in the lake play an important role in recovering nutrients and energy thrown off as waste by other organisms (see Section V), but their densities and levels of activity were never assessed before the studies described in this report. Even knowledge of the population sizes and distributions of fish in the lake is limited to order of magnitude approximations.

Our project, "Lower Trophic Level Interactions in the Pelagic Foodweb of Lake Champlain", was originally viewed as a project to fill in gaps in knowledge about the microbial portion of the lake's foodweb and to begin the process of developing a foodweb model for the lake. The Request for Proposals for the project asked that the research component include: measurement of productivity and biomass at every trophic level, quantification of energy and material transfer between trophic levels, and investigation of the impact of nutrient levels on all of these processes. The model was expected to be "predictive", allowing managers to rely on it to forecast the consequences of alternative management practices and thus ensure that wise choices are made.

While the information desired by the LCMC was indeed important information for understanding and modeling the lake's foodweb, accomplishing all of the suggested tasks would have required a multimillion dollar budget, a team of several scientists, and a long term research program. Since only \$95,000 over two years was available to fund the project, the proposal and work plan which was put forth by us, and accepted by EPA, was less ambitious, although still "in the spirit" of the RFP. It allowed for major steps forward in developing a dynamic picture of the lake's microbial foodweb..

The project involved five field and lab components, plus the modeling effort. Specifically the components and their individual goals were as follows:

1. Nutrient Enrichment Experiments

Nutrient enrichment experiments were conducted in experimental carboys in the main basin of the lake (Main Lake) for the purpose of identifying nutrient limitations among the lake's phytoplankton. This portion of the study was not explicitly requested by the LCMC. However, we (the investigators) felt that with interstate agreements on phosphorus controls in the works and a large portion of the LCMC budget being spent on analysis of P control methods and P dynamics in the Lake's watershed, the supposition of phosphorus-limited algal growth in the lake should be tested. The assays for nutrient limitation conducted by the EPA in the 1970's used *Selenastrum capricornutum* as a test organism. This alga is neither native to Lake Champlain, nor "average" in its nutrient requirements (it uses nitrogen and phosphorus at a higher ratio than most species; Rhee and Gotham 1980). While the analyses done were common practice at the time, the modern method for assessing nutrient limitations tests the native community under *in situ* conditions.

Our "follow-up" assessment was done *in situ*, using lakewater, and examined the possibility of N, P or Si limited algal growth, as well as mixed or changing limitations. We included Si in the study because diatoms are a major algal group in the lake and Si has been identified as a limiting factor for diatoms in Lake Michigan during summer (Schleske and Stoermer 1971). Nitrogen was included for two reasons: 1) because the literature shows that nitrogen limitation is almost as common in lakes as is phosphorus limitation (Elser et al. 1990), and 2) species of nitrogen-fixing bluegreen algae are common in Lake Champlain. Bluegreen algae are poor competitors for phosphorus and thus are scarce in P limited systems (Smith 1983). By contrast, they thrive during N scarcities, the reason being that they are able to extract N from the atmosphere while other algae cannot.

Identification of limiting nutrients through enrichment experiments is based on the fact that a limiting nutrient controls algal division rates so that the addition of a nutrient to an

experimental system containing algae will result in an increase in algal biomass (or chlorophyll a concentration) in that system, while enrichment with a nonlimiting nutrient will not. If only one nutrient is limiting, as is assumed for P in Lake Champlain, then addition of that nutrient in combination with other nutrients should result in the same biomass yield as when the nutrient is added on its own.

We conducted four nutrient enrichment experiments in the main basin of Lake Champlain (near Juniper Island), each during a different month of the growing season. Nitrogen, Si and P were added to duplicate carboys both singly and in all possible combinations, and the chlorophyll a concentrations and phytoplankton biomasses present after a 4-5 day incubation was assessed. Because different species of algae may be limited by different nutrients, we also examined algal response to nutrient additions at the division and species level.

2. Physiological Assays of Phytoplankton Phosphorus Status

Physiological assays of phytoplankton phosphorus status (alkaline phosphatase activity and orthophosphate turnover time) were conducted at five sites within the lake as additional evidence for or against widespread P limitation. This portion of the study was not paid for by the LCMC but by a HELIX grant to an undergraduate student (Staci Pomeroy) conducting an internship in the laboratory of Dr. Suzanne Levine. Alkaline phosphatase is an enzyme that cleaves phosphate groups off of organic compounds in the water. It is energetically expensive to produce and therefore is manufactured by algae in quantity only when they are P deficient. Orthophosphate (PO_4) is the form of phosphorus preferred by phytoplankton. During P limitation, algae deplete PO_4 supplies in the water so that concentrations fall to exceedingly low levels (typically a few nanograms per liter) and the turnover time of the PO_4 pool becomes very rapid (often 1-5 min; Wetzel 1985). Both substantial alkaline phosphatase activity in lake water and PO_4 turnover times of < 20 min are commonly used indicators of algal phosphorus deficiency.

3. Grazing Experiments

Three sets of grazing experiments were conducted at different times of the year (spring, summer and early fall) to permit estimation of the rates of carbon transfer between trophic levels. Specifically, we estimated zooplankton grazing on phytoplankton, heterotrophic protozoa, and bacteria, and zooplankton grazing on protozoa. For the phytoplankton, we determined grazing rates not just for the community as a whole, but also at the level of algal division and, when possible, for individual species. From our grazing experiments we also were able to obtain the first-ever estimates of bacterial productivity in Lake Champlain.

Grazing rates were estimated by creating a range of grazer densities in experimental systems (carboys) incubated *in situ* and measuring the resulting growth rates of the prey populations enclosed. Growth rates were estimated by sampling over a time course and regressing prey biomass against time. The next step in the calculations was regression of population growth rates on grazer densities: the slope of the equation obtained from this regression is an estimate of grazer clearance rates for the particular prey item (milliliters of water cleared of this prey per day per gram of predator). Finally, the product of clearance rate, predator biomass, prey biomass and carbon content of the prey yielded the estimate of C transfer that we desired. This method, introduced by Lehman and Sandgren in 1985, makes a number of assumptions: that sources of mortality other than grazing are unimportant or at least uniform in the experimental systems, that prey reproductive rate (unlike their death rate) is independent of predator density, and that the species composition of the phytoplankton and zooplankton communities is maintained through the manipulations required to change grazer densities. We added nutrients to the experimental systems to saturate nutrient-limited algal and bacterial growth and thus eliminate the possible effects of nutrient excretion by zooplankton on the growth of algae and bacteria. Different densities of zooplankton should yield different levels of nutrient recycling, and thus could lead to a gradient in reproductive rates were the systems not overwhelmed with outside nutrient. The other assumptions of the method were examined

through careful monitoring of organisms. We report in Sections IV and V about some observed violations of the assumptions.

Because we were interested in grazing by different types of grazers, our grazing analyses included 3 subsets of carboys. In one subset, macrozooplankton (animals retained by a 202 μm sieve; mostly cladocerans and adult and copepodite copepods) were manipulated to three density levels; in another microzooplankton (animals sized 20-202 μm ; mostly copepod nauplii and rotifers) densities were similarly manipulated and in the third, we produced two density levels of protozoa (animals < 20 μm in size). Reductions in grazer density were achieved through sieving, and enhancements through adbacks of either sieved or netted animals. The manipulations were done sequentially, so that the microzooplankton treatments were free of macrozooplankton (or should have been) and the protozoan treatments free of microzooplankton.

Bacterial production was estimated from the y-intercepts of the bacterial growth rate versus grazer density regressions. Because productivity was determined in the presence of added nutrients, it may well be an overestimate of actual bacterial production in the lake. Conducting separate estimates of bacterial productivity using tritiated thymidine would have been useful, however, we did not have the substantial additional funds required to do so.

4. Primary Production Measurements

Primary productivity was measured for the Main Lake site during two of the grazing experiments using the ^{14}C technique and an incubator which exposed algal samples to a range of light intensities. A numerical model (Fee 1990) was used to combine information on algal photosynthetic response to light, with data on solar irradiance during the course of the experiments, and light extinction rates in the lake, to arrive at estimates of daily primary production rates.

In addition, the parameters of the light response curves calculated by the model runs provided information on the light status of phytoplankton in the lake. For algae that conduct much of their photosynthesis at low light intensities, the slope of the initial part of a measured

light response curve (α) is steep, while P_{\max} , the maximum rate of photosynthesis achieved at optimal light intensities, and I_k , the light intensity at which photosynthesis begins to saturate (i.e., is no longer light limited) are normally low. In short, these algae are efficient at using small amounts of light, but are wasteful of light when it is plentiful. By contrast, algae accustomed to higher light intensities have light-response curves with low α , high I_k and high P_{\max} . They specialize at efficient harvesting of light at high light intensities. Most important for our interests, light limitation of primary productivity is suspected whenever I_k is less than the average light intensity of a lake's mixed layer (I_{ave} ; Fee 1990).

5. The Nutrients versus Grazers Experiment

The final experiment conducted involved simultaneous manipulation of nutrients and macrozooplankton in a 2 X 3 factorial design. Undertaken in July 1995, this experiment's purpose was to examine the relative strength of nutrients and grazers as potential controllers of prey densities and to reveal any interactions between the two variables. The principal response variables were bacterial and protozoan densities, and chlorophyll a concentration. Chlorophyll a was measured as a "quick and cheap" alternative to phytoplankton counting. However, since we recognized that grazing rates could be estimated from the fertilized portion of this experiment, we analyzed phytoplankton species densities in fertilized carboys at the beginning and end of the study. Our principal approach to analyzing the relative importance of nutrients, grazers and their interaction was two-way ANOVA.

6. The model

The purpose of the numerical model developed was to organize thoughts about interactions within the pelagic community and their impact on normal seasonal dynamics. Because foodwebs are tremendously complex, it is difficult for scientists to anticipate or understand all the repercussions of changes in nutrient availability or in the biomass or activity of foodweb components without the aid of a model. Models need to be kept simple to facilitate

analysis. The goal is to capture important details and ignore those details that are unlikely to greatly influence conclusions. Our model focuses on carbon and phosphorus flow between trophic levels (it assumes that P limits algal growth), and, in its current form, emphasizes phytoplankton and zooplankton. Thus the following components of the foodweb are modeled as explicit compartments: phytoplankton C and P contents, herbivorous zooplankton C and P contents, carnivorous zooplankton C and P contents, and the concentration of P in the epilimnion, hypolimnion, and sediments. Additional components of the foodweb (fish, benthic organisms, bacteria and protozoa, as well as excreted C) are included in the model indirectly, in the sense that many of their effects are taken into account, but there are no model compartments dedicated to their representation. These components may be added to the model as their dynamics become better defined. Another limitation of the current model is that it does not yet allow for useful extrapolation to multiple years.

The model is a set of first-order nonlinear differential equations. A model run starts with given initial conditions (i.e., starting values for the state variables defined by the person running the model). External driving functions also need to be defined. The output of a model run is a data file that can be looked at in any text editor or spreadsheet application. It can be displayed graphically through a utility or within a spreadsheet application.

Even with only a small number of system components in the model, a multitude of parameters need numerical values for the model to run. For example, for phytoplankton, information is required on nutrient uptake and growth dynamics, respiration, sinking rates and grazing mortality. Unfortunately, most of the parameter values required are not well known for Lake Champlain. Thus, it is necessary to use values obtained for other lakes or to make back-of-the-envelope calculations for Lake Champlain based on sketchy information to run the model. Consequently, it would be inappropriate to refer to the model as a "Lake Champlain Model" at this time. As more research is done on the lake, however, model parameter values can be altered to "fit" the model to it.

Findings of the Experiments Related to Nutrient Limitation in Lake Champlain

None of the four enrichment studies conducted in 1994 and 1995 suggested strict P limitation of phytoplankton growth in the main basin of Lake Champlain. During the spring study (May 1995), no nutrient combination (even N, Si, and P added together) elicited any growth response from phytoplankton, suggesting "unlimited" phytoplankton growth (growth tethered only by the mechanics of cell division and protoplasm production) or growth limitation by a factor not included in the analysis (most likely, light). At this time, the lake was undergoing vernal overturn (mixing down to 70 m at our sampling site) and a massive diatom bloom was underway.

The June 1994 experiment yielded very different results from the May study. Addition of either N or P to carboys stimulated growth, while addition of the two nutrients in combination yielded a response roughly equal to the sum of the separate responses. The simplest interpretation of this outcome was that mixed N and P limitation prevailed among the phytoplankton. Most likely some phytoplankton species were limited by N and others by P.

In July and September, yet another limitation scheme was apparent. Nitrogen added on its own had no impact on algal growth while singular P addition led to either no or a mild increase in phytoplankton biomass. The two nutrients had to be added together to increase phytoplankton biomass substantially. This outcome can be interpreted as indicating one of any three situations: 1) phosphorus limitation accompanied by near limitation by nitrogen, so that P addition quickly brought on N limitation; 2) phytoplankton growth that is in equilibrium with natural N and P inputs and thus "limited" by both; or 3) limitation by a third, unmeasured factor (such as light or grazers) which was alleviated by the experimental conditions, allowing phytoplankton to respond to the N + P additions with increased growth. In any case, P was not strongly limiting.

None of the enrichment experiments suggested that Si is limiting in the Lake: diatoms did not respond to Si addition even in midsummer when Si concentrations are at their lowest.

Taxon-specific responses to nutrient addition were not discernable in these experiments. Green algae, diatoms, and cryptophytes were the groups which increased in biomass under N+P fertilization, but these groups also tended to increase (at the expense of bluegreen algae) in control carboys. Hence their selection as dominants may have been related to conditions created in the carboys (e.g., higher light intensity or reduced grazing pressure relative to what they were exposed to in the lake).

The physiological indicators of P status yielded information supportive of the conclusion that P is not consistently a limiting nutrient in the lake. While orthophosphate turnover times (OTTs) were <20 min, and thus indicative of P deficiency, at Shelburne and Malletts Bays during midsummer, the measurements made in September were longer. The other three sites examined, St. Albans Bay, Burlington Harbor, and our site on the Main Lake, consistently had OTTs > 20 min, and thus appeared to have phosphorus-sufficient algal communities. The shortest OTT measured at the Main Lake site was, in fact, a full 80 minutes.

Alkaline phosphatase activity (APA) was measured on only two occasions in 1995. Significant activity was measured at all sites but St. Albans Bay in July-August 1995, suggesting inorganic P shortages at this time. However, very little APA was measured in September. Again the suggestion was alternating P sufficiency and deficiency at various sites around the lake. St. Albans Bay, however, never showed signs of P deficiency.

Findings of the Grazing Experiments

Grazing on Phytoplankton

The grazing experiments indicated that both large and small zooplankton play a role in controlling phytoplankton biomass in Lake Champlain. Although the rotifers and copepod nauplii that made up the microzooplankton in our experiments represented substantially less biomass than macrozooplankton (cladocerans and adult and copepodite copepods) in the lake during our studies, our estimates of macro and microzooplankton grazing rates were of a similar order of magnitude. This was because the micrograzers were more efficient (had higher

clearance rates) on a per animal weight basis. Clearly rotifers must be included in zooplankton monitoring programs on the lake. Up until the current monitoring program of New York State, zooplankton surveys routinely ignored these animals.

The experiment verified the findings of earlier studies on other lakes which suggested taxonomic variability in phytoplankton vulnerability to grazers (e.g., Porter 1973; Lehman and Sandgren 1985). Green algae and dinoflagellates appeared to be more vulnerable to macrograzer consumption than cryptophytes, and cryptophytes more vulnerable than blue green algae and diatoms. Micrograzers avoided green algae but fed on members of the other groups. Analysis of grazing rates at the species level indicated that even within the phytoplankton divisions, there is a great deal of variability in the edibility of different members. For example, *Cryptomonas* sp. was grazed at a rate which was on average four times the grazing rate experienced by its cousin *Chroomonas* sp. Among the bluegreen algae, *Aphanizomenon flos-aqua* was more heavily grazed than Chroococcales, and the diatom *Melosira* sp. was more seriously grazed than species of *Fragilaria* and *Tabellaria*. The differential edibility of algal species suggests a mechanism for phytoplankton species selection and community structuring that has not received a great deal of attention. During the last decade, resource availability and resource competition have been the backbone of phytoplankton succession theories (e.g., Tilman et al. 1982).

The daily loss rate of phytoplankton to grazers (both micro and macrozooplankton) during the three grazing experiments varied from 2-20% of standing stocks. The C involved was equivalent to just 3% of primary productivity in September 1994, but accounted for the entire lot (117%) in May.

Grazing on Bacteria and Protozoa

It has been only in the last twenty years that aquatic scientists have come to realize the abundance and energy contribution of bacterioplankton. Once considered important only in nutrient remineralization, aquatic bacteria are now known to number on the order of 10^6 per milliliter and contain 5-25% of the carbon fixed by photosynthetic algae. Estimates of

bacterioplankton productivity in lakes differ widely, but there is a consensus that the bacterial community divides rapidly and turns over quickly. Production rates are typically $1-100 \times 10^6$ cells·L⁻¹h⁻¹, the rate dependent on depth, season, and nutrient status.

In Lake Champlain bacterioplankton abundance was in the range of $1-2 \times 10^6$ cells·mL⁻¹ and bacterial productivity was on the order of $10-45 \times 10^6$ cells·L⁻¹h⁻¹. Bacterial abundance and production varied by season. Based on just the four grazing experiments conducted for this study, bacterioplankton population dynamics in Lake Champlain appear similar to those of other lakes.

Assuming that the bacterial population size was stable over several weeks, as it is in most aquatic systems, then the entire bacterial community was being replaced every 2 to 7 days. From another perspective, given that aquatic bacteria contain about 1×10^{-14} g carbon per cell and the lake contains approximately 2.6×10^{16} mL, then every 2 to 7 days 5.2×10^8 grams or 1.1×10^6 lbs of carbon are moving from bacteria to someplace else. That someplace else is likely to be ingestion by protozoan and invertebrate grazers.

There was at least one occasion, for each grazer size category considered in this study, macro-, micro-, and nanograzers, when bacterial ingestion could be measured. Macrograzers had measurable bacterivory ($1.1 \text{ mL} \cdot \mu\text{g dry wt}^{-1} \cdot \text{d}^{-1}$) when cladocerans were dominant. Cladocerans are known to harvest particles the size of bacteria, and when they are present, cladocerans can provide a one-step trophic link between tiny fast-growing bacteria and much larger zooplanktivorous fish. Micrograzers had measurable grazing rates when rotifers were dominant, another group known to be capable of ingesting bacteria-sized particles. Nanograzers, specifically heterotrophic protozoa, are considered to be the primary bacterivores in aquatic systems, but we could obtain measurable rates only once. This may have been due to inadequate replication, or some other factor that masked nanograzer bacterivory. As with the bacterial abundance and productivity data, bacterivory rates measured in Lake Champlain are in line with measurements in other lakes, and therefore, the literature should provide additional information on rates that would be useful in future modeling efforts of the Lake Champlain food web.

Macrozooplankton and micrograzers were found to ingest heterotrophic protozoa with clearance rates that ranged from 0.02 to 4.2 mL· μ g dry wt⁻¹·d⁻¹. As with feeding on bacteria, cladocerans and rotifers appeared to be the two taxa responsible for feeding on protozoa. Given that heterotrophic protozoa feed on bacteria, zooplanktivorous fish could be linked by two trophic steps to bacteria via cladocerans and rotifers feeding on heterotrophic protozoa. The key organisms that connect the microbial loop to the macroorganism foodweb in Lake Champlain are rotifers and cladocerans. When these grazer taxa are abundant, human perturbations that affect fish will affect bacteria and vice versa.

Primary Productivity in the Lake

The primary productivity measurements that we made in September 1994 and May 1995 were the first ever for the lake. They indicated a rate of production typical of a mesotrophic lake: 1660 mg C m⁻²·d⁻¹ in May and 349 mg C m⁻²·d⁻¹ in September. Overall productivity was greater in May than September because the phytoplankton standing stock was greater and so was daily solar irradiance. Volumetric primary production rates, which were obtained by dividing the areal rate by the depth of mixed layer, were similar: 24 and 19 mg C m⁻³·d⁻¹ in May and September, respectively.

The parameters of the photosynthesis-light relationship suggested phytoplankton populations adapted to low light availability (α was high and P_{\max} low. This was not surprising given that the lake was mixing down to 70 m in May and to 18 m in September. During both experiments, $I_k:I_{ave}$ values for the lake were well below 1, suggesting that primary production was light limited. While no primary production measurements were made during our summer studies, the depth of the mixing zone was essentially the same as the depth of the photic zone (10 m). Thus we suspect that light availability is an issue for Lake Champlain's phytoplankton even in summer.

Findings of the Nutrients vs. Grazers Experiment

Addition of nutrients (N, P and glucose) to experimental carboys during the final experiment fairly consistently stimulated growth, regardless of zooplankton treatment. Thus two-way ANOVA indicated that nutrient level accounted for a substantial portion of experimental variability in chlorophyll concentrations and bacterial densities.

The grazing portion of the study was more difficult to interpret. ANOVA indicated a *positive* response of phytoplankton to zooplankton biomass. This response was weaker than that to nutrients, but still significant at a $p = 0.06$. A positive phytoplankton response to grazers might arise if nutrient recycling by zooplankton is substantial and increases as grazer density increases (as it must). In a sense, this argument reinforces the earlier conclusion that nutrients are a critical determinant of algal biomass. On the other hand, a positive growth response to grazers could be related to herbivore-carnivore interactions in the carboys. Cyclopoid copepods, which feed on rotifers, nauplii, and smaller cladocerans, were the dominant zooplankton present during this experiment. The potential for these predators to affect herbivorous zooplankton dynamics was most apparent in our carboys with low macrozooplankton densities and nutrient amendments. Nutrients stimulated algal growth, and rotifers took advantage of the greater food supply to increase in numbers (see Fig. 7; Section IV). Cladocerans also may have been affected by the manipulations of carnivorous zooplankton. While cladocerans were removed along with cyclopoids from our "low macrozooplankton" treatments, and thus could not benefit from the improved algal food availability in these systems, there clearly were more cladocerans in the carboys with the ambient level of macrozooplankton (and thus cyclopoids) than in those at the "high" level.

Correlation analysis provided more insights into the complicated relationships between zooplankton and phytoplankton populations. The results of the analyses done were very much dependent on whether carboys were fertilized or not. Examining only fertilized systems, we found both chlorophyll a concentration and phytobiomass to be negatively correlated with herbivore biomass (the biomass of zooplankton other than cyclopoids). Under unfertilized

conditions, a more complicated relationship was indicated: chlorophyll a concentrations increased with herbivore biomass at low herbivore levels, but declined at high levels (phytobiomass wasn't measured). When correlation analysis was done considering all zooplankton (herbivores and carnivores), a positive relationship between chlorophyll a and this parameter when carboys were unfertilized, but no relationship when they were fertilized. What these results suggest is that both nutrient recycling by grazers and grazing mortality play a role in determining phytoplankton biomass. In nutrient poor systems, the importance of the former may outweigh that of the latter. Cyclopoids are beneficial to algae as they both recycle nutrient and remove herbivores.

For the bacterioplankton, the nutrient versus grazer experiment showed control by both nutrients (N, P and C) and grazers in late summer. The effects were unequivocal and highly significant statistically. The two factors were interactive, consistent with studies that have shown that the degree of grazer control on bacterioplankton depends on the trophic status of the system. Heterotrophic protozoa were controlled by just grazers. The increase in bacteria from added nutrients did not increase heterotrophic protozoa, suggesting that their growth is not resource limited.

The most surprising result of this experiment was the length of the trophic cascade that occurred after manipulation of macrozooplankton abundance. At first, macrozooplankton enhanced bacterial growth by suppressing heterotrophic protozoa. Then later, after macrozooplankton reduced rotifers, and rotifer predation on heterotrophic protozoa declined allowing the protozoan populations to rebound, bacteria declined quickly. Macrozooplankton predation on rotifers appeared to govern, in opposite directions, the growth of heterotrophic protozoa and bacteria. The experiment demonstrated how it is conceivable that manipulating one group of large organisms, like piscivorous fish, can have consequences for the smallest organisms, bacteria.

Model Status and Characteristics

The values of the following model parameters were found to be particularly important in driving model behavior: minimum phytoplankton nutrient quota; maximum phytoplankton rate of growth; and both zooplankton (herbivorous and carnivorous) growth (grazing) and death (and excretion) rates. It is these parameters that determine whether model behavior produces a quick or a slow transition towards equilibrium (following disturbance) and whether oscillations develop. Thus, changes in zooplankton species composition brought on by management actions (such as fish stocking) could have significant indirect effects. On the other hand, the values of the following parameters had relatively little effect on system behavior: the concentration of dissolved, available forms of nutrients (P) in the water, as compared with the threshold for phytoplankton uptake; the exact rate and efficiency of nutrient uptake by the phytoplankton; the temporary storage of excess nutrients inside the phytoplankton; and the exact rate of nutrient release from detritus.

With some parameter values, the model runs approach an equilibrium over time. With other parameter values, the model exhibits periodic behavior. With realistic initial conditions and parameter values, the equilibrium or the regular oscillations do not arise until after a time interval that represents more than one growing season. Given that the winter mixing of the lake, and other seasonal effects, perturb the system in major ways, we can expect the system to spend the bulk of each growing season in transient behavior. The transient dynamics in our model are not very sensitive to initial conditions. Parameter values have a far stronger effect on the shape of the curves.

With appropriate parameter values, the model runs produce the pattern typical of the lake: a spring bloom of phytoplankton, followed by an increase in herbivorous zooplankton which in turn drives the phytoplankton abundance down for a "clear water phase". After that the zooplankton usually decrease, pinched between the decline in food availability and an increase in predation pressure by the carnivores. Real-world details such as the phytoplankton species composition would undoubtedly modify the actual lake behavior, especially late in the season.

Net primary production in the model shows an interesting pattern: even as the abundance of the phytoplankton varies greatly over time in most runs, their aggregate productivity varies to a far lesser extent.

The model could be extended in many ways, to exclude details found of lesser importance, and to add details thought important (e.g., epilimnetic detritus, and associated bacterial and protozoan activity). Zooplankton predation by fish could be fleshed out to include seasonal variation. But most importantly, in order to develop a model that has the ability to predict responses of the Lake Champlain system to management actions, more detailed data are needed. The available estimates of critical parameters, such as grazing rates, are too imprecise, and limited to specific spots at specific times. Given the size and variability of Lake Champlain, getting the necessary data is a challenging and expensive undertaking. Modeling should proceed alongside further measurements, as the improvement in model usefulness will be gradual and will feed back into the planning of experimental studies.

Conclusions and Management Implications

Our experiments indicated a highly dynamic and interactive foodweb in Lake Champlain. Nutrients, grazing and light all appear to play a role in regulating the standing stocks and community structure of the phytoplankton, while nutrients and grazing control bacterial densities. Primary productivity in the lake is at a level typical of a mesotrophic lake. Carbon flow from algae to upper trophic levels occurs through zooplankton feeding on phytoplankton, and also through the microbial loop. At least at some times during the summer, the microbial loop seems to be well connected to the classic foodweb through zooplankton feeding on heterotrophic protozoa. The greatest flow of C from bacteria to zooplankton, however, occurs when cladocerans are present, as these animals are efficient bacterivores, as well as consumers of algae. The model suggested that the foodweb may be particularly sensitive to the nutrient uptake dynamics of phytoplankton, to sinking and grazing rates, and to nutrient recycling via animal

excretion and upwelling from the hypolimnion during seiches. Further research should be conducted on these processes.

Understanding Lake Champlain well enough to predict the consequences of management practices is a worthy and wise goal. It is one that will take a continued effort by a diversity of experts, however, as well as a steady level of funding for on-lake activities.

References

- Elser, J.J., Marzolf, E.R., and Goldman, C.R. 1990. Phosphorus and nitrogen limitation of phytoplankton growth in the freshwaters of North America: A review and critique of experimental enrichments. *Can. J. Fish. Aquat. Sci.* 47: 1468-1477.
- Fee, E.J. 1990. *Computer programs for calculating in situ phytoplankton photosynthesis*. Can. Tech. Rep. Fish. Aquat. Sci. 1740: v+27.
- Fee, E.J., Shearer, J.A., BeBruyn, E.R., Schindler, E.U. 1992. Effects of lake size on phytoplankton photosynthesis *Can. J. Fish. Aquat. Sci.* 49: 2445-2459.
- Lehman, J.T., and Sandgren, C.D. 1985. Species-specific rates of growth and grazing loss among freshwater algae. *Limnol. and Oceanogr.* 30: 34-46.
- Porter, K.G. 1973. Selective grazing and differential digestion of algae by zooplankton. *Nature* 224.
- Rhee, G.-Y., and Gotham, I.G. 1980. Optimum N:P ratios and coexistence of planktonic algae. *J. Phycol.* 16: 486-489.
- Schleske, C.L., and Stoermer, E.F. 1971. Phosphorus, silica and eutrophication of Lake Michigan. *Spec. Sympos. Amer. Soc. Limnol. Oceanogr.* 1: 157-171.
- Smith, V.H. 1983. Low nitrogen: phosphorus ratios favor dominance by blue-green algae in lake phytoplankton. *Science* 221: 669-671.
- Spencer, C.N., McClelland, B.R., Stanford, J.A. 1991. Shrimp stocking, salmon collapse, and eagle displacement. *BioScience* 41: 14-21
- Tilman, D., Kilham, S.S., and Kilham, P. 1982. Phytoplankton community ecology: The role of limiting nutrients. *Ann. Rev. Ecol. Sys.* 13: 349-372.
- Wetzel, R.G. 1983. *Limnology*. Philadelphia: Saunders College Publishing.

SECTION II

GENERAL INTRODUCTION

Lake Champlain is an important resource for the states of Vermont and New York and the province of Quebec. Every year hundreds of thousands of people visit the lake to fish, boat, swim or otherwise enjoy its waters. Others come to view the shipwrecks, the forts and other historical sites. Visitors spend an estimated \$2 billion dollars per year in the Lake Champlain Basin; thus the lake is clearly a focus of the region's economy. The lake's value extends far beyond its ability to attract tourists, however. It is a source of drinking water for hundreds of thousands of Vermonters and New Yorkers and a place of solace for them. Most importantly, though, Lake Champlain is a thriving ecosystem, the home of over 80 species of fish, and a diversity of invertebrates, plants and algae.

While Lake Champlain is generally considered a "healthy" lake, it is far from pristine in condition. Excess nutrient inputs have led to algal blooms and dense weed beds in some parts of the lake, and toxic compounds are present at high enough concentrations throughout the lake that advisories against the consumption of trout and walleye have been in effect during the past two years. Chemical inputs have not been the only sources of stress, however. Several biological invasions have taken place, aided by the St. Lawrence and Lake Champlain canals: Eurasian milfoil, water chestnut, sea lamprey and zebra mussels are examples of plants and animals that now thrive in the lake at the expense of native species. In addition, stocking of the lake with nonnative sports fish has been a common practice, with consequences for native fishes and lower trophic levels that are largely unknown.

Management of Lake Champlain has focused on phosphorus reductions to control plant and algal biomass and on fish stocking and lamprey control to sustain the sports fishery. Citizen groups, such as the Lake Champlain Committee, the New York and Vermont Citizens Advisory Committees on Lake Champlain, and the Lake Champlain Monitoring Program, demonstrate a public interest in lake stewardship, while the federal government has shown its support for wise management of the Lake by providing monies for research and planning through the Lake

Champlain Special Designation Act of 1991. State government commitment is evident in the recent agreement between the two states to reduce phosphorus inputs to tributaries.

Successful lake management depends on a familiarity with lacustrine biological communities and with the environmental factors that influence ecosystem structure and function. Particularly important is knowledge of foodweb structure and of controls on primary productivity. A lake's foodweb has as its nodes all of the biological species present in a lake and as the threads between nodes feeding relationships. Energy and materials initially incorporated by plants and algae move through foodwebs along "food chains" to ultimately support fish production. Foodwebs are complex: dozens of species may exist at one trophic level and compete with one another for shared resources, animals may use a diversity of food sources, and many species affect others indirectly by modifying the environment (e.g., by releasing nutrients or, conversely, growth-inhibiting chemicals). Furthermore, foodwebs contain detrital and bacterial components that are often ignored, but which may recycle large amounts of nutrient and organic matter which otherwise would have been lost to the system (this part of the foodweb is referred to as "the microbial loop"). Because of all the linkages in foodwebs, perturbation at one node of a foodweb may yield a proliferation of effects. Effects may move up, across, or down foodwebs, or in multiple directions.

Management with incomplete information about a lake and its foodweb is ineffective and can lead to "surprises" that may at times be disastrous. An example of a management effort that rebounded in an unexpected manner was *Mysis* (opossum shrimp) introduction to Rocky Mountain lakes (Spencer et al. 1991). The intent of the shrimp introductions was to provide the principal sports fish, kokanee salmon, with an additional food source and thus improve its productivity. However, most of the Montana lakes were deep, so that *Mysis* was able to escape fish predation by hovering above the lake bottom in the dark during the day and coming to the surface at night to feed on zooplankton. In Flathead Lake, Montana, the natural food of the kokanee salmon was the zooplankton on which the *Mysis* fed. Because *Mysis* proved to be the superior competitor for zooplankton, the end result of the mysid introduction to this lake was

collapse of the kokanee fishery. Repercussions of the introduction did not end here, however: eagles and grizzly bears accustomed to feeding on the fish left the area in search of new food supplies. With no fish to catch or wildlife to watch, tourists stopped coming to the lake. Ecological horror stories in other lakes have included the loss of natural fish stocks following intentional introductions of nonnative game fishes (e.g., Zaret and Paine 1973; Moyle et al. 1986).

For Lake Champlain, there is concern that the two main management goals, larger piscivorous fish populations and reduced algal and macrophyte biomass, might interfere with one another. Increased piscivorous fish populations might, for example, reduce zooplanktivorous fish densities to the point that zooplankton populations will be relieved of predatory controls. Phytoplankton might respond to the resulting increase in zooplankton grazing in either of two ways: with reduced populations (yielding greater water clarity), or by species shifts towards large inedible species immune to grazers. The blue green algae that form surface scums are in the latter group. On the other hand, reduced P inputs might lead to lower energy transfer up the foodweb, with the result that the lake's fish carrying capacity might be reduced.

Lake Champlain is a woefully understudied lake for its size and its recreational and historical importance. Only during the last five years have zooplankton and phytoplankton densities in the lake been monitored on a regular basis, while natural (nonpathogenic) bacterial and heterotrophic protozoan densities have never been assessed lakewide. The only estimates of the importance of these components of the microbial loop are those presented in this report. Primary productivity is another critical variable that to our knowledge was never measured in the lake before the study described here. While phosphorus control is an important part of the Lake's management, and especially of watershed management activities, minimal testing of nutrient limitation in the lake has taken place. Assays for nutrient limitation conducted by EPA during the 1970's used an exotic species (*Selenastrum capricornutum*) as a test organism (US EPA 1974; Myers and Gruendling 1974). While this practice was common at the time, modern

studies of nutrient limitation are conducted *in situ* using the native phytoplankton community. The current report describes the outcome of the first assays of this sort.

Our project, "Lower trophic level interactions in the pelagic foodweb of Lake Champlain", was funded by the U.S. EPA through the Lake Champlain Management Conference to provide some of the missing information on microbial foodwebs required for effective management of Lake Champlain. Because of the very limited funds available (\$95,000), the project was restricted to the Main Lake and to two growth seasons. Specifically, the project's objectives were:

1. To assess the relative importance of phosphorus, nitrogen, and silica as limiting factors for phytoplankton, taking into account possible variations in limitation at the species level and possible changes in limitation type over the course of a growth season;
2. To estimate primary productivity in the lake on 2-3 occasions (the budget was far too small for a seasonal analysis, even at one site);
3. To estimate the grazing rates of zooplankton feeding on phytoplankton and the relative "edibility" of different phytoplankton types;
4. To examine the strength of the "microbial loop" in Lake Champlain by measuring macrozooplankton and protozoan grazing rates on bacteria, as well as macrozooplankton feeding on protozoa (effectively linking the "loop" to the classic herbivore-based food chain);

5. To develop a simple model of the lower levels of the foodweb in Lake Champlain.

Because so little is known about the growth dynamics and sources of mortality for Lake populations, the model's principal function could not be to predict lake response to disturbance (as the LCMC hoped it would be). Its realized function was to identify and prioritize future research needs for the lake.

That our effort was very small relative to the research effort needed to understand and effectively manage Lake Champlain and its foodweb cannot be overemphasized. The analyses that we performed should be extended to other basins of the lake and should be repeated

throughout the growth season, and over multiple years. Lakes do not behave identically from year to year, but fluctuate over a range of conditions that can only be defined through long term studies. Normal ranges of conditions must be known if the effects of disturbance are to be detected.

A historical shortcoming of research on Lake Champlain has been inadequate dissemination of results. Researchers have used "grey literature" reports and local presentations as their principal means of reporting results. Consequently, as these researchers have retired and the few copies of their reports have dropped out of circulation, valuable data have been lost. Desire to break away from this pattern has prompted us to format this report in a manner which will lead to rapid dissemination of our findings to peer-reviewed journals. The report is organized into six chapters: this introduction, individual chapters on four topics central to the study (nutrient limitation in the lake, the effects of zooplankton grazing on phytoplankton, Lake Champlain's "microbial loop", and the foodweb model), and a final discussion relating our findings to questions raised by the Management Conference in their request for proposals. The chapter on nutrient limitation is "in press"; it will appear in Volume 23, Issue 2 of the *Journal of Great Lakes Research*. The two papers on zooplankton-phytoplankton interactions and the microbial loop are early drafts that we plan to submit to journals this summer. We do not intend to publish the model of Lake Champlain's foodweb until more of the parameters required to run the model are available for the lake.

References:

- Gruendling, G.K. 1974. Nutrients, phytoplankton, and limiting factors in Lake Champlain, New York and Vermont., *Conference on Water Research on Lake Champlain*, Vermont Water Resources Research Center, pp. 39-49.

- Moyle, P.B., Li, H.W., Barton, B.A. 1986. The Frankenstein effect: impact of introduced fishes on native fishes in North America. In *Fish Culture in Fisheries Management*, ed. R.H. Shroud, pp. 415-426. New York: Springer-Verlag
- Spencer, C.N., McClelland, B.R., Stanford, J.A. 1991. Shrimp stocking, salmon collapse, and eagle displacement. *BioScience* 41: 14-21
- U.S. EPA. 1974. *EPA National Eutrophication Survey. Report on Lake Champlain, New York and Vermont, EPA regions I and II*. Corvallis, OR: Pacific Northwest Environmental Research Lab.
- Zaret, T.M., Paine, R.T. 1973. Species introduction in a tropical lake: A newly introduced piscivore can produce population changes in a wide range of trophic levels. *Science* 182: 449-455.

SECTION III

Phosphorus, Nitrogen and Silica as Controls on Phytoplankton Biomass and Species
Composition in Lake Champlain (USA-Canada)

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ABSTRACT

The long-standing assumption that the phytoplankton in Lake Champlain are phosphorus limited was tested through measurement of physiological indicators of phosphorus status (alkaline phosphatase activity and orthophosphate turnover time) and enrichment experiments conducted four times during the growth season. Phosphorus addition to experimental carboys incubated 4-5 days *in situ* substantially increased phytoplankton biomass relative to controls in June, but had only a mild impact in July and September, and no effect in May. Nitrogen addition augmented biomass in one of four experiments (in June), while silica had no impact at any time. In summer and fall, addition of N and P in combination always yielded more phytoplankton biomass than singular P addition. In spring, even combined addition of N, P, and Si failed to stimulate phytoplankton growth. The phytoplankton groups responding to fertilization were largely the same as those that flourished in controls (diatoms and green algae), suggesting that enclosure was a more powerful determinant of species composition than nutrient inputs. Orthophosphate turnover times and levels of alkaline phosphatase activity in the lake indicated spatial and temporal variability in phytoplankton P status, with P sufficiency as common as P deficiency. We conclude that multiple interacting factors influence the abundance and species composition of phytoplankton in Lake Champlain. In spring, phytoplankton growth is not limited by N, Si or P, but by some factor yet to be determined (perhaps light or temperature). In summer, P is the principal limiting nutrient, but N exerts an influence that deserves further investigation.

Index words: Lake Champlain, phytoplankton, phosphorus, nitrogen, silica, limiting nutrient, alkaline phosphatase activity, orthophosphate turnover time

INTRODUCTION

Lake Champlain is the largest lake in the northeastern United States aside from the Great Lakes. Named in 1609 by explorer Samuel Champlain, it has a colorful history of Revolutionary War sea battles, commercial fishing and shipping, log runs, lakeside industry, and recreation. Stresses on the lake are reminiscent of those that have altered the Great Lakes: early overfishing, followed by extensive stocking with non-native fish species, inadvertent introductions of exotic plants and animals (e.g., Eurasian milfoil, water chestnut, sea lamprey, white perch, and most recently, the zebra mussel), and pollution with heavy metals, organic compounds and nutrients. For residents of the Lake Champlain Basin, the alteration of greatest concern has been lake eutrophication. The main lake basin (referred to as the Main Lake), while still no worse than mesotrophic in condition, has become noticeably more turbid in recent decades. More seriously, some shallow portions of the lake (St. Albans Bay, Mississquoi Bay, and the river-like southern end) have developed summer algal blooms and dense macrophyte beds. In an attempt to slow down eutrophication, the states of Vermont and New York and the province of Quebec have twice entered into agreements aimed at reducing P inputs to the lake. In addition, federal monies have been appropriated through the 1990 Lake Champlain Special Designation Act to support research on P transport and retention in the Basin, the impact of land use on P runoff, and the planning of control strategies. Oddly, a basic premise underlying all these activities is yet to be thoroughly tested: the assumption that P, and only P, limits phytoplankton growth in Lake Champlain.

Two lines of evidence have been used to argue for P limitation in the lake: TN:TP ratios in the Main Lake that generally range between 65:1 and 110:1 by moles (Walker 1986; VT DEC and NY State DEC 1994; lower ratios are found in some bays, the Northeast Arm and South Lake), and thus are greater than the Redfield ratio for average phytoplankton nutrient content (15:1; Redfield 1958), and the outcome of growth limitation (Provisional Algal Assay Procedure) bioassays performed by the U.S. EPA and

local cooperators in 1972-74 (U.S. EPA 1974; Gruendling 1974). Both arguments have weaknesses. The first assumes that N and P are made available to phytoplankton via lake inputs and recycling in the same ratio at which they are present in standing stocks, and that all forms of N and P, including detritus and dissolved organic compounds, are bioavailable, premises that have been repeatedly refuted in the literature (e.g., Harris 1986; Howarth 1988; Levine and Schindler 1992). Although a general relationship between nutrient limitation type and TN:TP ratio has been demonstrated (e.g., Morris and Lewis 1988; Downing and McCauley 1992), the relationship is not strong enough for predictive use. Smith (1982) has noted that TN, as well as TP, influences chlorophyll a concentrations up to a TN:TP ratio of 77:1. He has also shown that there is a relationship between dominance by blue-green algae, which are generally favored by N deficiencies, and TN:TP ratio (Smith 1983). Significantly, blue green algal dominance is not limited to lakes with TN:TP ratios <15:1, but occurs in lakes with TN:TP ratios as high as 64:1. These findings suggest that TN:TP ratios often exaggerate the bioavailability of N pools. The cause may be refractory N in terrigenous organic material, or poorer foodweb recycling of N than of P.

Like many studies done at the time, the bioassays for nutrient limitation conducted by the U.S. EPA on Lake Champlain in the 1970's used *Selenastrum capricornutum* as a test organism. *S. capricornutum* is neither native to Lake Champlain nor a species with a typical critical N:P demand ratio (it uses N and P at a higher N:P ratio than most phytoplankton; Rhee and Gotham 1980). Therefore, it is unclear how well this species represented the response that Lake Champlain's phytoplankton community would have had were it exposed to excess nutrients. Nor were the results of the bioassays as conclusive about P limitation in the lake as generally believed. *S. capricornutum* responded to P but not N addition when added to waters from western portions of the lake (including the main basin), and thus appeared to be P limited in these waters. However, it grew much more when N and P were added in combination than

when P was added alone. Furthermore, in waters from the Northeast Arm of the lake and St. Albans Bay, *S. capricornutum* appeared to be co-limited by N and P, and N limited, respectively.

In this paper, we reassess the issue of nutrient limitation in Lake Champlain, with our focus on conditions in the Main Lake. A new assessment was deemed necessary because: 1) twenty years have passed since EPA's assessment; 2) we feel that *S. capricornutum* results should not be relied upon to represent limitations of natural phytoplankton assemblages in the lake; 3) EPA's finding of frequent synergistic N plus P effects in *S. capricornutum* bioassays suggests a secondary role for N in phytoplankton biomass regulation in the lake; and 4) heterocystous (i.e., potentially N₂-fixing) blue-green algae are found throughout the lake in late summer. While blue-green algal scums may be blown into the Main Lake from more eutrophic lake segments, we cannot dismiss the possibility that these populations develop in place. Their presence hints at episodes of N deficiency in the lake.

Another motive for the study was concern that the common practice of classifying lakes as P limited, or limited by some other single factor, may oversimplify reality. The practice assumes that the many phytoplankton coexisting in a lake are uniform in their resource requirements and acquisition abilities, and thus conform as a single entity to Liebig's Law of the Minimum. While studies in the laboratory (Rhee 1978; Rhee and Gotham 1980; Terry 1980) have shown that most algal species switch abruptly between N and P or Si and P limitation at specific N:P or Si:P supply ratios (critical demand ratios) without passing through a stage of simultaneous N+P or Si+P limitation, there is considerable interspecific variability in the ratios at which these switches occur. For example, the critical Si:P demand ratios of diatoms range from 6:1 to 527:1 (Tilman 1982), while species-specific critical N:P demand ratios vary at least between 7:1 and 49:1 (Rhee and Gotham 1980; Tilman *et al.* 1982). Thus it is distinctly possible that phytoplankton communities, if not individual species, experience periods of mixed

resource limitation. Resource competition theory exploits this supposition to explain phytoplankton species diversity and succession in lakes (e.g., Tilman 1992; Tilman *et al.* 1992). By tracking the responses of individual phytoplankton taxa to nutrient additions during our study, we hoped to unveil species- or group-specific differences in nutrient limitation type among the phytoplankton of Lake Champlain. Such information would promote better understanding of phytoplankton dynamics in the lake.

Our experimental approach was a combination of 1) enrichment experiments *in situ* using natural phytoplankton assemblages and water, and testing for N, P and Si limitation; and 2) measurement of physiological indicators of P status (alkaline phosphatase activity (APA) and orthophosphate turnover time (OTT)). The former analysis was done for a single site in the Main Lake; the latter, at five sites (four in western parts of the lake and one in eutrophic St. Albans Bay). Si was included in the enrichment studies because of its reported importance as a limiting nutrient for diatoms in Lake Michigan (Schleske and Stoermer 1971). The physiological assays provided supplementary information on the severity of phytoplankton P shortages at the site of the enrichment experiment, and also permitted assessment of spatial variability in phytoplankton P status. The two lines of investigation addressed somewhat different types of nutrient limitation: the physiological measures aided in identifying short-term limitations on cell enlargement and division rates (Blackman limitation), while the enrichment experiments integrated growth and loss responses over 4-5 days to assess limitations on algal yield (Liebig limitation). An excellent review of these two limitation types and their ecological implications can be found in Cullen (1991).

SITE DESCRIPTION

Lake Champlain straddles the border of Vermont and New York and extends a short distance into Quebec (Figure 1). Formed through glacial gouging, the lake has been in the

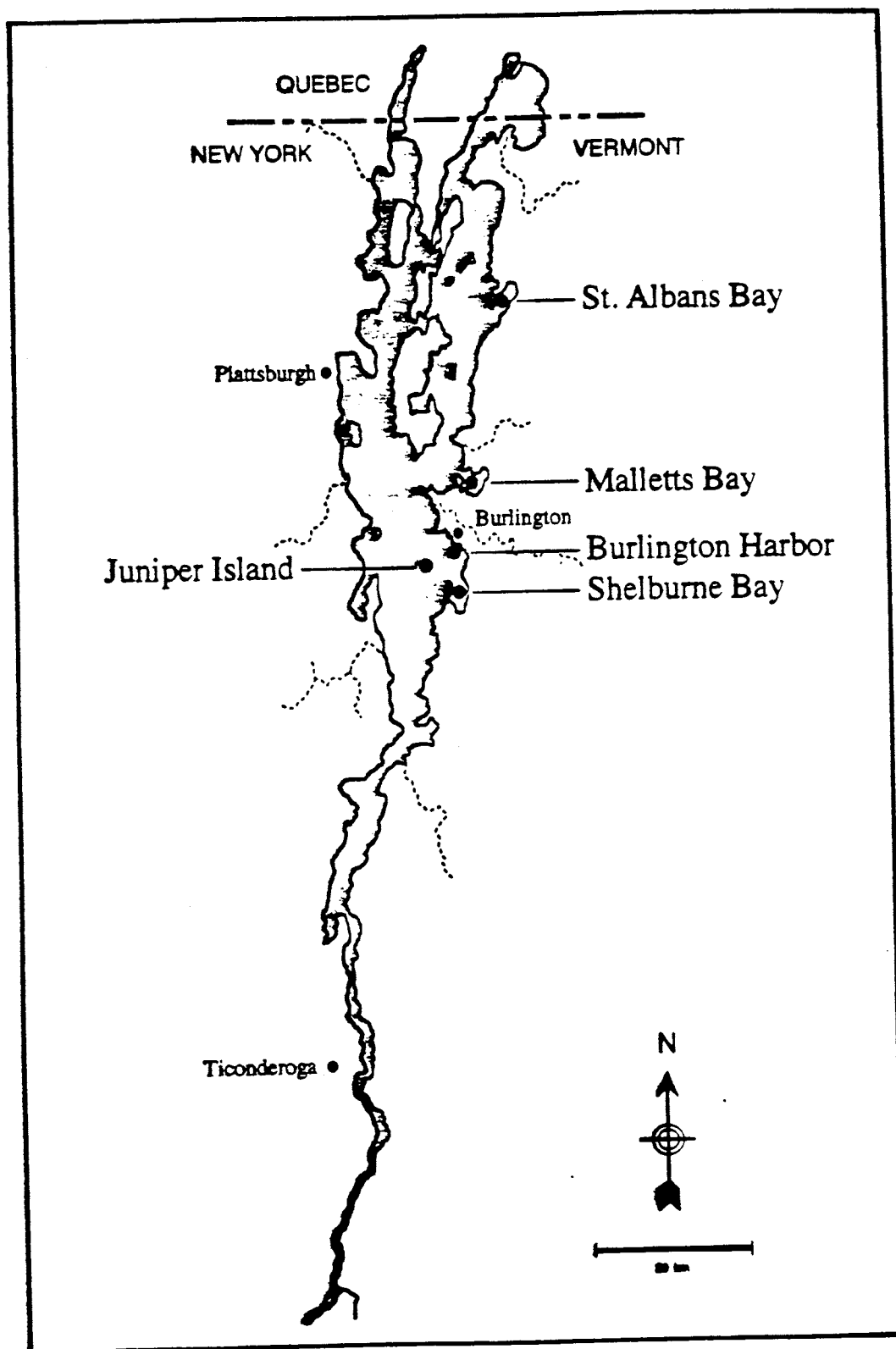


Figure 1. Map of Lake Champlain identifying the sampling sites.

past both a large proglacial lake and an inland sea. Currently long (170 km), narrow (maximum width, 20 km) and deep (maximum depth 122 m; mean depth 23 m), the lake has a surface area of 1,130 km² and a volume of 26 km³. Numerous islands, sills, peninsulas and causeways divide the lake into segments with somewhat different chemical and biological characteristics. More than 300 tributaries flow into Lake Champlain, draining 19,881 km² of largely forested and agricultural land. The lake empties to the north through the Richelieu River, a tributary of the St. Lawrence River, but is also connected at its southern end to the Hudson River via the Hudson-Champlain Canal. Mean lake water residence time is 2.9 years.

Because of its size, the main basin of Lake Champlain mixes deeply in summer (mean epilimnion depth, 10-12 m), and prevailing winds along the main axis of the lake (from the south) result in a frequent large amplitude internal seiche (T. Manley, pers. comm., Middlebury College, 1996). The shallow south end of the lake and most bays are ice covered for about four months in winter, but the main basin generally freezes for only 1-2 months (sometimes not at all).

The phytoplankton community of the main lake is typically dominated by diatoms and cryptophytes, although these species are frequently replaced by blue-green algae (along with some green algae and dinoflagellates) in late summer and autumn (Myer and Gruendling 1979, McIntosh *et al.* 1993). In the southern shallow end of the lake and in St. Albans Bay, blue green algae frequently dominate over much of the summer. Common zooplankton in the lake include the cladocerans *Bosmina longirostris*, *Daphnia retrocurva*, and *Daphnia galeata mendota*, the copepods *Mesocyclops edax*, *Diacyclops bicuspidatus thomasi*, *Diaptomus minutus* and *Diaptomus silicis*, and the rotifers *Keratella cochlearis*, *Polyarthra major*, *Kellicottia longispina* and *Notholca* sp. (McIntosh *et al.* 1993) More than 80 species of fish are present (Myer and Gruendling 1979).

The sampling site for our enrichment study was at the broadest expanse of the lake, about 1 km northwest of Juniper Island (Fig. 1). Sampling for alkaline phosphatase activity

and phosphate turnover time was done at Shelburne Bay, Burlington Harbor, St. Albans Bay, and Malletts Bay, as well as at the Juniper Island site. Data on the morphometry and nutrient conditions of the sampling sites are given in Table 1.

METHODS

Enrichment Experiments

Enrichment experiments were conducted during early (June 9-13), mid (July 18-22), and late (September 15-20) summer 1994, and again in spring (May 15-19) 1995. Environmental conditions at the sampling site during the experimental times are summarized in Table 2.

Ten-liter gas-permeable polyethylene carboys were used as experimental units. These were filled with water and phytoplankton collected from a depth of one meter, using either a centrifugal pump (June and July) or an 8-L van Dorn bottle (September and May). No effort was made to remove zooplankton from the collected waters as we wished to include their recycling of nutrients in the assay. The water was mixed in a 240 L polypropylene tank prior to carboy filling. Eight treatments were carried out in triplicate: controls (no nutrient amendment), singular amendments of P ($3.2 \mu\text{M KH}_2\text{PO}_4$), N ($17.9 \mu\text{M NH}_4\text{Cl}$), or Si ($17.8 \mu\text{M Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$), and amendments of N, P and Si in combination (N+P, N+Si, P+Si, and N+P+Si). Nutrient amendments increased TP concentrations by 6-12 fold, dissolved inorganic nitrogen (DIN) concentrations by 2-3 fold, and SiO_4 concentrations by 0.4-7 fold (with the greatest enrichment in midsummer). The carboys were incubated in outer Burlington Harbor for 4-5 days suspended from anchored floating frames at a depth of 1.5 (last 3 experiments) or 2 m (first experiment). Midday light intensities at the incubation depth were generally below the threshold for photoinhibition for most phytoplankton groups ($<1000 \mu\text{mol m}^{-2} \text{s}^{-1}$), yet high enough ($>300 \mu\text{mol m}^{-2} \text{s}^{-1}$) to saturate photosynthesis (Reynolds 1984).

Table 1

At the end of the incubation period, the carboys were retrieved, shaken, and sampled in duplicate for chlorophyll *a* and phytoplankton. Phytoplankton samples were preserved with 1% acid Lugol's solution, and chlorophyll samples were kept on ice in the dark during transport to the laboratory.

Within five hours of collection, chlorophyll samples (0.2-1 L) were filtered onto Whatman GFF glass fiber filters (effective pore size 0.7 μm) and the pigments extracted in hot ethanol (Sartory and Grobbelaar 1984). The filters were allowed to leach an additional 24 h in the dark, after which the chlorophyll extract was centrifuged to remove suspended particles, and chlorophyll *a* absorbance was read on a spectrophotometer using the monochromatic procedure with phaeophytin correction (Lorenzen 1967). Initially, we waited 10 min between adding acid (0.005 N HCl) to chlorophyll extracts and reading phaeophytin absorbance. Later it was discovered that a 30 min incubation allowed for a more complete shift of chlorophyll *a* absorbance out of the 665 nm band. Although we reached similar conclusions about chlorophyll response to treatment using chlorophyll values with and without the correction for phaeophytins, the uncorrected values were more variable and thus judged untrustworthy. Consequently, some of the chlorophyll *a* data presented here (Fig 2, June and July) are not corrected for phaeophytin.

Algal species composition and cell densities were determined by direct microscopic counts of settled samples using an Olympus inverted light microscope. Cells that appeared unhealthy (ruptured, color faded, or infested with bacteria) were not counted. The biovolume of each phytoplankton species was estimated from its cell dimensions and geometry (Wetzel and Likens 1991). For common species, cell dimensions were measured during every incubation and in all treatments; scarce species were measured during at least one incubation. Algal biomass was estimated from biovolume using a carbon:volume conversion factor of $0.1 \mu\text{g C} \cdot \mu\text{m}^{-3}$ (Wetzel and Likens 1991).

One-way analysis of variance (ANOVA) was used to test for treatment effects and a Duncan multiple-range test (Sokal and Rohlf 1981) to examine the statistical significance of differences between treatment means.

Orthophosphate Turnover Time

Orthophosphate turnover time (OTT) was estimated by adding ^{32}P -labelled orthophosphate to 200 mL of sample in a stirred beaker at ambient temperature and withdrawing 10 mL aliquots for filtration over a time course (Levine and Schindler 1980). To distinguish phytoplankton uptake from combined bacterial and phytoplankton uptake, the analyses were done using two filter pore sizes, 0.2 μm (bacteria + phytoplankton) and 1 μm (phytoplankton only). Rate constants for phosphate uptake (k) were obtained from the exponential regression of percent ^{32}P activity in sample filtrate versus time. Only the initial loglinear portion of the uptake curve was used in the estimation of k . OTT is equal to $1/k$.

OTT decreases in lakes as phytoplankton become progressively more P deficient, both because PO_4^{3-} concentration is reduced to a miniscule amount and because enzyme affinity for P becomes very high (Cembella *et al.* 1984). Establishing a critical boundary between OTTs indicative of P sufficiency versus those suggesting P deficiency is an arbitrary process, but values of 20 min (Lean and Pick 1981), 40 min (White *et al.* 1985), and 60 min (Waiser and Roberts 1995) have been suggested. We compared our OTT measurements with all of these criteria.

Alkaline Phosphatase Activity

Alkaline phosphatase activity (APA) was measured using the procedure of Petterson (1980). 3-0-methylfluorescein phosphate was added to 4 mL aliquots of lakewater and the development of 3-0-methylfluorescein product measured over a 1 h time course with a Turner 110 fluorometer. Samples were buffered with TRIS to pH 8.3 (optimum pH for APA; also close to the mean pH for Lake Champlain, 8.2) and maintained at ambient temperature

in a constant temperature bath during the incubation. To normalize APA to phytoplankton biomass, 1-2 L aliquots of the collected water were filtered through Whatman GFF filters and analyzed for chlorophyll *a* using the procedure described above.

APA < 0.05 nmol $\mu\text{g chl } a^{-1} \text{ min}^{-1}$ was deemed “constitutive” (related to the loss of protoplasmic AP from damaged cells), while greater APA was attributed to exoenzyme production in response to inorganic P shortages. APA in excess of 0.2 nmol $\mu\text{g chl } a^{-1} \text{ min}^{-1}$ was considered indicative of serious P deficiency (Pettersson 1980; Smith and Kalff 1981; Gage and Gorham 1985; Jansson *et al.* 1988; Guildford *et al.* 1994)

RESULTS

Enrichment Experiments

Each of the four enrichment experiments had a somewhat different outcome, reflecting seasonal differences in environmental conditions and in phytoplankton assemblages.

May Experiment

The experiment in May 1995 took place while the lake was turning over, diatoms were blooming, and the temperature was just 4°C. None of the treatments, N, Si or P addition, alone or in combination, yielded a significant increase in chlorophyll *a* concentration or in phytoplankton biomass (Fig. 2; one-way ANOVA indicated a lack of treatment effect at $p = 0.05$). Community dominance by diatoms also was maintained (Fig. 3). The only change observed was a minor one: cryptophyte and blue-green algal populations, already small in the lake, diminished further in the carboys, while green algae increased to replace them (Figs. 3, 4). This succession was observed in control and treated carboys alike, suggesting an enclosure effect.

June Experiment

The June 1994 experiment took place at a higher TP concentration and a lower TN:TP ratio than the other enrichment studies (Table 2). This experiment was difficult to

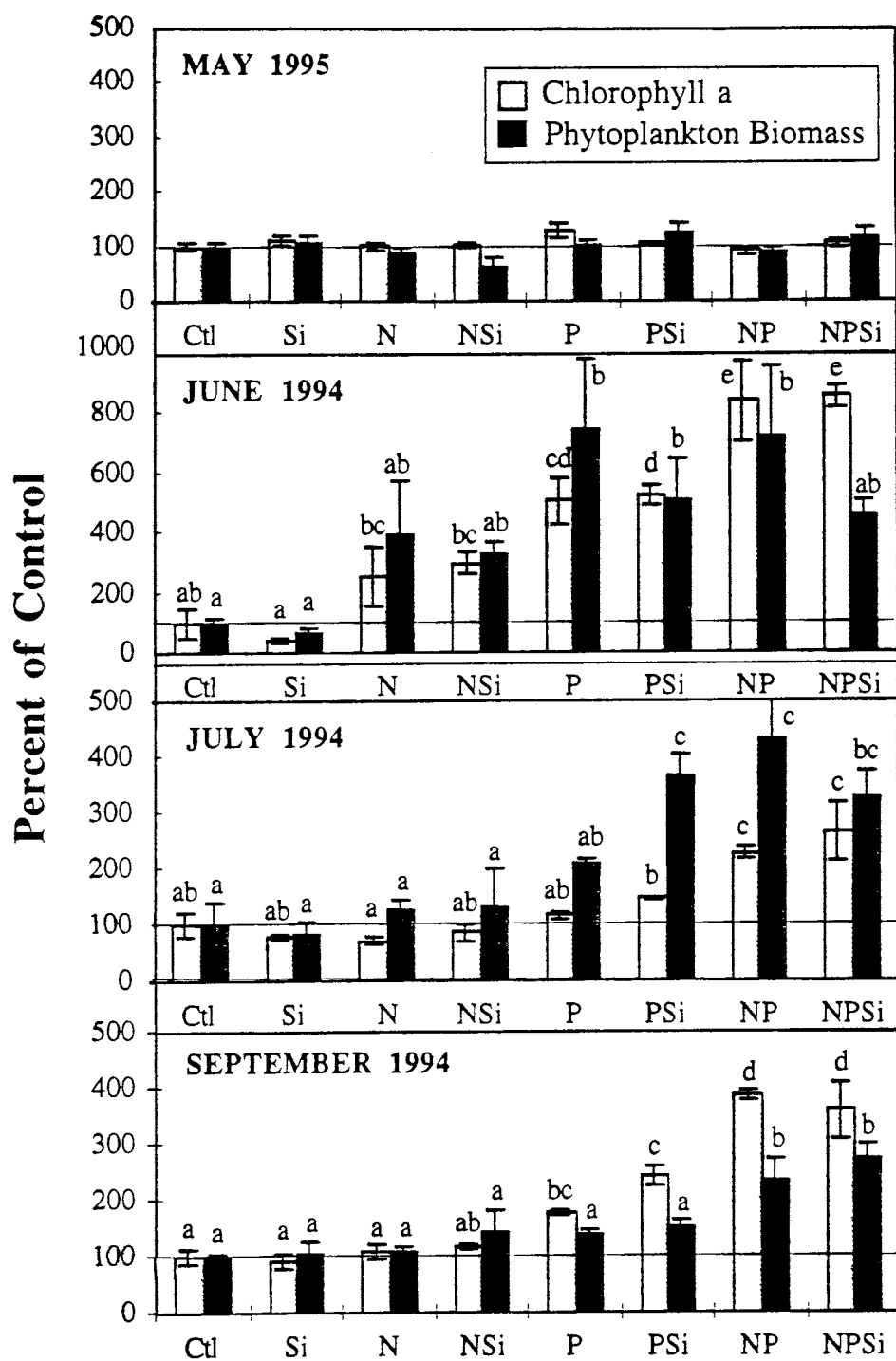


Figure 2. Chlorophyll *a* concentration and phytoplankton biomass in carboys treated with N, Si, and P, alone or in combination, after 4-5 days incubation *in situ*. The columns show treatment means as percentages of the control mean; the bars, standard error. The horizontal line is a reference to control levels. The letters above columns indicate whether treatment means are significantly different from one another ($p = 0.05$; Duncan's multiple range test); a shared letter indicates no significant difference. No letters are assigned to the May values as ANOVA indicated no treatment effect.

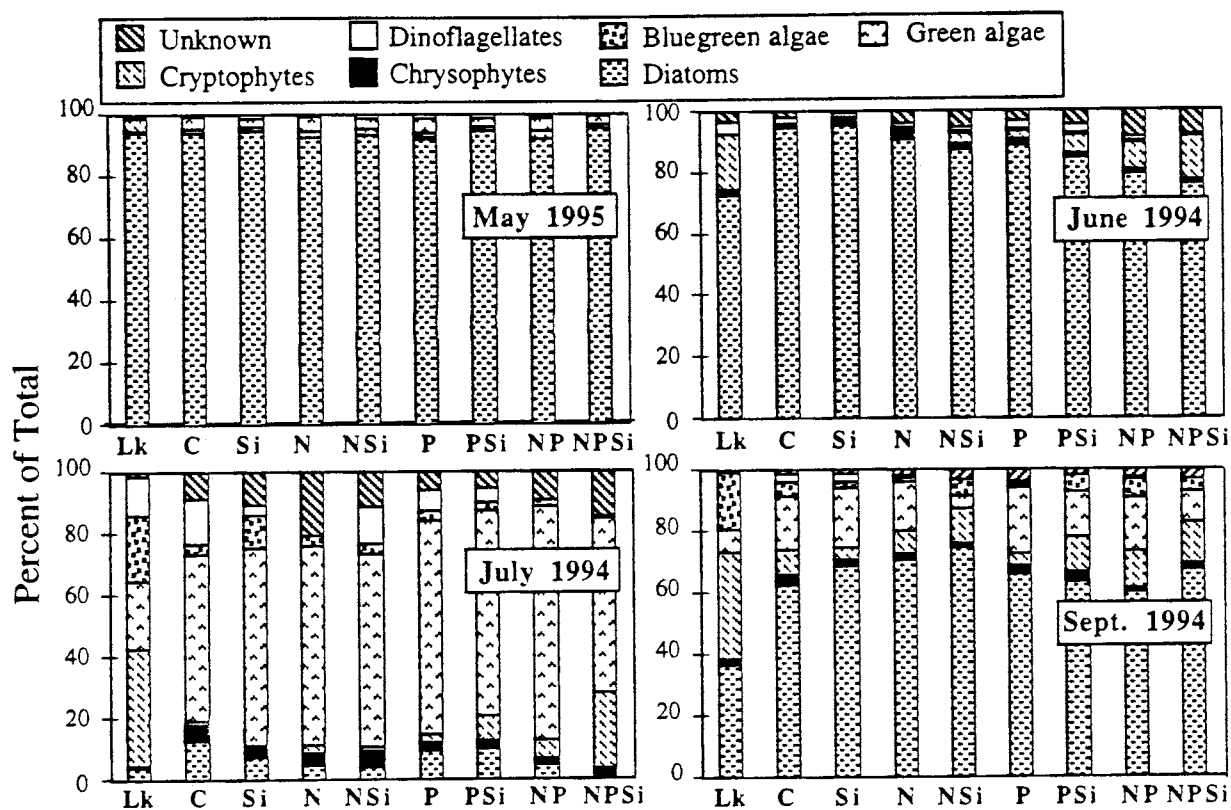


Figure 3. The phytoplankton species composition (relative abundance by group) in the experimental carboys after 4-5 days incubation, compared with that in the inoculum added to carboys (LK). Euglenophytes were very rarely seen and thus not plotted.

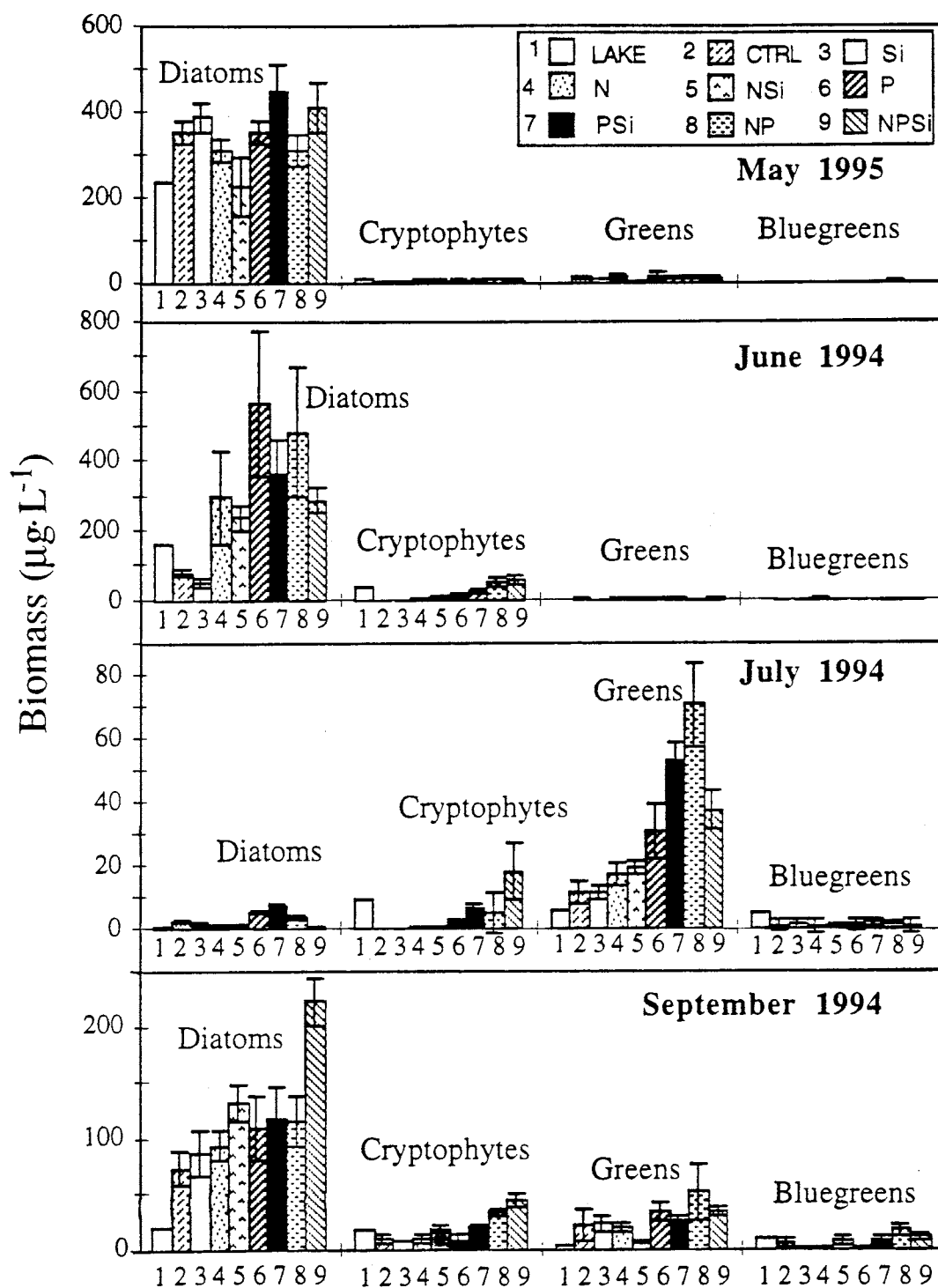


Figure 4. Comparison of the biomass of four phytoplankton groups in the water added to experimental carboys (L) with that in carboys treated with N, Si, or P, alone or in combination, and allowed to incubate in the lake for 4-5 days. Untreated control carboys (C) are also included. Column heights indicate mean values in $\mu\text{g L}^{-1}$; the bars, standard errors about means. Chrysophytes and dinoflagellates were also present, but generally at lower densities.

Table 1. Characteristics of the five sampling sites. Median values are given for the ice-free periods of 1992-1995, except in the case of Shelburne Bay, which was not sampled during this period. For it, the values are for 1990-1991. All data are from the Vermont DEC, except those in brackets, which are from the NY State DEC.

Characteristic	Burlington Harbor	Juniper Island	Malletts Bay ¹	Shelburne Bay	St. Albans Bay
Depth (m) ²	15	100	32	25	7
TP (μM)	0.36	0.39	0.26	0.33	0.71
SRP (μM)	0.06	0.16	0.06	-	0.13
TN (μM)	29	32	29	-	31
DIN (μM) ³	10	16	14	-	1
TN/TP	82	83	113	-	43
DIN/TP	28	41	53	-	2
DSi (μM)	14	21	77	-	20
Chlorophyll a ($\mu\text{g L}^{-1}$) ⁴	2.9 (5.3)	1.9 (4.8)	2.1 (3.5)	2.7	3.5 (6.7)
Secchi Depth (m)	4.9	5.2	4.8	4.5	2.5

¹ Malletts Bay is divided into inner and outer sections by a causeway. We sample the inner bay, DEC the outer.

² Depths given here are those at the DEC sites. Our sites were up to a kilometer away, and thus may have differed in depth. For example, our Juniper Island site was at a depth of 70 m.

³ NH_3 was consistently below the detection limit, 1.4 μM . These values are for nitrate+nitrite-N.

⁴ The VT DEC sampled over just one season; the NY DEC over the entire period.

Table 2. Conditions at Juniper Island at the times of our enrichment studies. Water temperature and epilimnion depth were measured during carboy filling. The other data were collected by the Vermont DEC, generally within a week of our study. May sampling by the DEC was done two weeks before our experiment, however.

Characteristic	June 1994	July 1994	Sept. 1994	May 1995
Temperature ($^{\circ}\text{C}$)	12	21	17	4
Epilimnion Depth (m)	11	12.5	21	70
Secchi Depth (m)	3.6	5.3	6.5	-
TP (μM)	0.52	0.32	0.29	0.36
TDP (μM)	0.10	0.13	0.13	0.19
TN (μM)	35	29	38	49
DIN (μM)	14	11	9	-
TN:TP	68	88	130	139
DIN:TP	27.7	35.4	32.0	-
DSi (μM)	46	3	25	-

interpret because it coincided with a period of phytoplankton decline in the lake. After four days of incubation, phytoplankton biomass and chlorophyll *a* concentrations in the control carboys averaged just 38 and 19% of initial values (Fig. 5). Consequently, although we observed large responses to P and N enrichment relative to controls (Fig. 2), these responses reflect the poor survival of phytoplankton in controls as much as true increases in treatment carboys. Superposition of treatment effects on changing background conditions also may account for high standard errors in the data.

Despite these limitations, the data suggest that both P and N played a role in phytoplankton control at the time of this experiment. Carboys receiving only P had chlorophyll *a* concentrations and total phytoplankton biomass levels 5-7 times the levels in controls, while the N-only carboys supported levels augmented 2-4 fold (Fig. 2; the latter increase was not statistically significant at $p = 0.05$ due to high standard errors). Maximum chlorophyll *a* and phytoplankton biomass levels were attained when P and N were added in combination. Si addition had no apparent impact on phytoplankton biomass, despite community dominance by diatoms.

Several changes in phytoplankton community composition were observed. Although the bulk of the biomass loss observed in control and Si carboys was related to declining diatom populations (Fig. 4), cryptophytes suffered greater proportional losses. Thus diatoms were more strongly dominant at the experiment's end than at its beginning in these carboys (Fig. 3), despite decreased densities. The principal species to respond to enrichment (whether with N, P or N+P) were diatoms. Cryptophytes also increased slightly in abundance under combined N+P treatment, but the impact of singular additions of N or P was to moderate the rate of cryptophyte (especially *Chroomonas* sp.) decline rather than to stimulate net growth (Fig. 4).

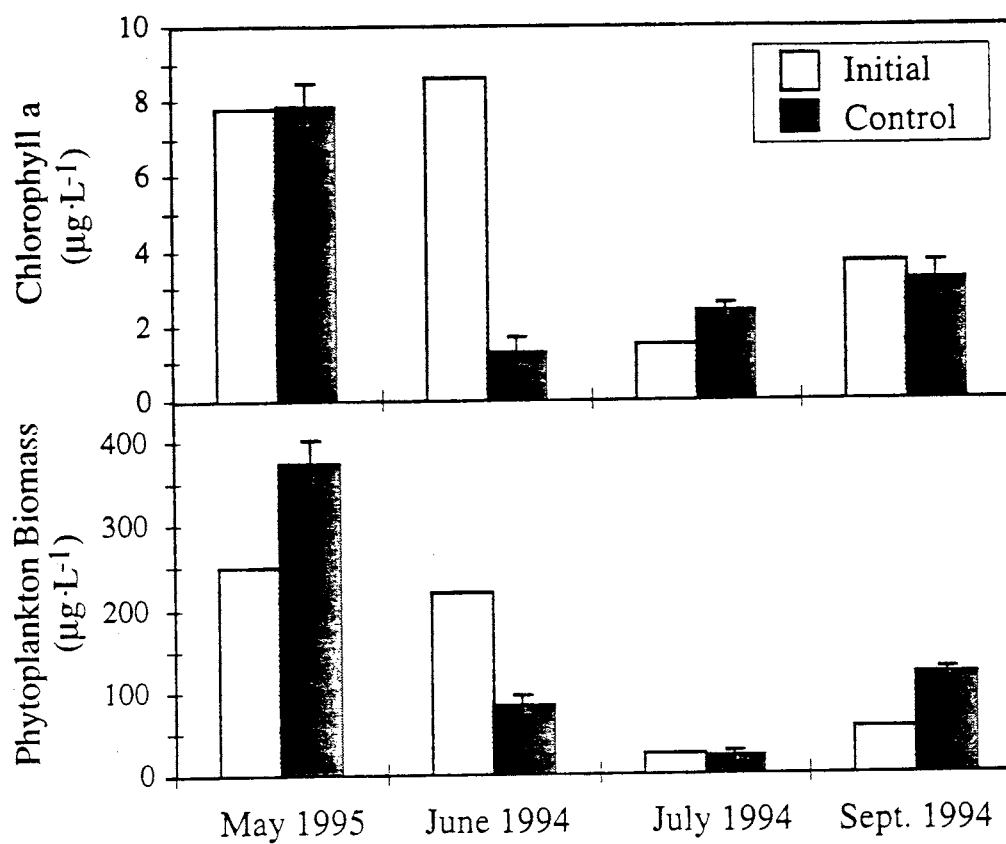


Figure 5. Comparison of chlorophyll *a* concentration and phytoplankton biomass in control carboys incubated 4-5 days *in situ* with the initial values for the water added to the carboys.

July Experiment

The July experiment was conducted during the "clear water" phase in Lake Champlain. Chlorophyll *a* and phytoplankton biomass were at levels more typical of an oligotrophic than a mesotrophic lake (Fig. 5). Dissolved silica concentration was exceptionally low (2.8 μM) and diatoms scarce. Dominance in the phytoplankton community was shared by a mix of green algae, cryptophytes, and blue-green algae (Fig. 3). N did not stimulate phytoplankton growth when added on its own during this experiment, nor did Si (Fig. 2). Singular P addition yielded an increase in total phytoplankton biomass that was not quite large enough to be statistically different from the control (which had a high standard error), and no increase in chlorophyll *a* concentration. Larger phytoplankton biomass and chlorophyll *a* accruals took place when P was added in combination with either Si or N. For these treatments too, phytoplankton biomass responded more strongly than chlorophyll *a*, suggesting that newly-produced organisms required less chlorophyll than their progenitors.

Phytoplankton community composition changed dramatically in both the control and fertilized carboys during the July experiment (Fig. 3). Both cryptophytes and blue-green algae were greatly reduced in biomass in most carboys, while the green algae became more abundant (Fig. 4). Enrichment with P in combination with either Si or N moderated the decline in cryptophytes and N+Si+P enrichment actually stimulated cryptophyte growth. Blue-green algal growth, however, was not augmented by any of the nutrient treatments. Most of the increase in phytoplankton biomass observed in enriched carboys was associated with green algae, especially species of *Eudorina* and *Oocystis*.

September Experiment

By the September experiment, the phytoplankton community in Lake Champlain had returned to a mix of diatoms and cryptophytes, with blue-green algae the third most well represented group (Fig. 3). TP concentration was similar to that in July, but TN and DSi

concentrations were greater. In this experiment, as in July, N and Si addition had no impact on phytoplankton levels, while P addition elevated chlorophyll *a* concentrations, but not phytoplankton biomass (Fig. 2). Again, the strongest growth response was to the combined addition of N and P. In September, chlorophyll *a* concentration was more sensitive to nutrient addition than phytoplankton biomass, a trend opposite to that observed in July. Apparently, cells directed more energy into new pigment production for increased photosynthesis than into cell division.

Cryptophytes, green algae and, especially, diatoms (*Tabellaria* sp., *Fragilaria crotonensis*, *Asterionella formosa*, and an unidentified small centric species) responded positively to enrichment during this experiment (Fig. 4). An enclosure effect was also observed: cryptophytes and blue green algae declined in the control carboys, while green algae and diatoms increased.

Phosphate Turnover Times

Orthophosphate turnover times in Lake Champlain (Fig. 6) were found to be somewhat long compared with those reported for a diversity of P-stressed lakes in the literature (<10 min in summer; Rigler 1964; Peters 1975; Peters and MacIntyre 1976; Levine and Schindler 1980; White *et al.* 1985). OTTs of less than 60 min were found during mid- and late summer in Malletts Bay, Shelburne Bay and Burlington Harbor, suggesting P deficiency among phytoplankton according to the criterion of Waiser and Robarts (1995). The former two sites also had OTTs below Lean and Pickett's (1981) more stringent critical threshold of 20 min; the shortest turnover time measured for Lake Champlain was 8 min. Some of these same sites had turnover times >> 60 min duration when sampled in early summer or fall, however, and OTTs <60 min in duration were never measured for St. Albans Bay or the Juniper Island site. While too little data were collected to clearly define temporal trends, it appears that the general seasonal pattern for OTT in the lake is a maximum in spring, followed by a decline over summer and a return to somewhat longer OTTs as the thermocline degrades in

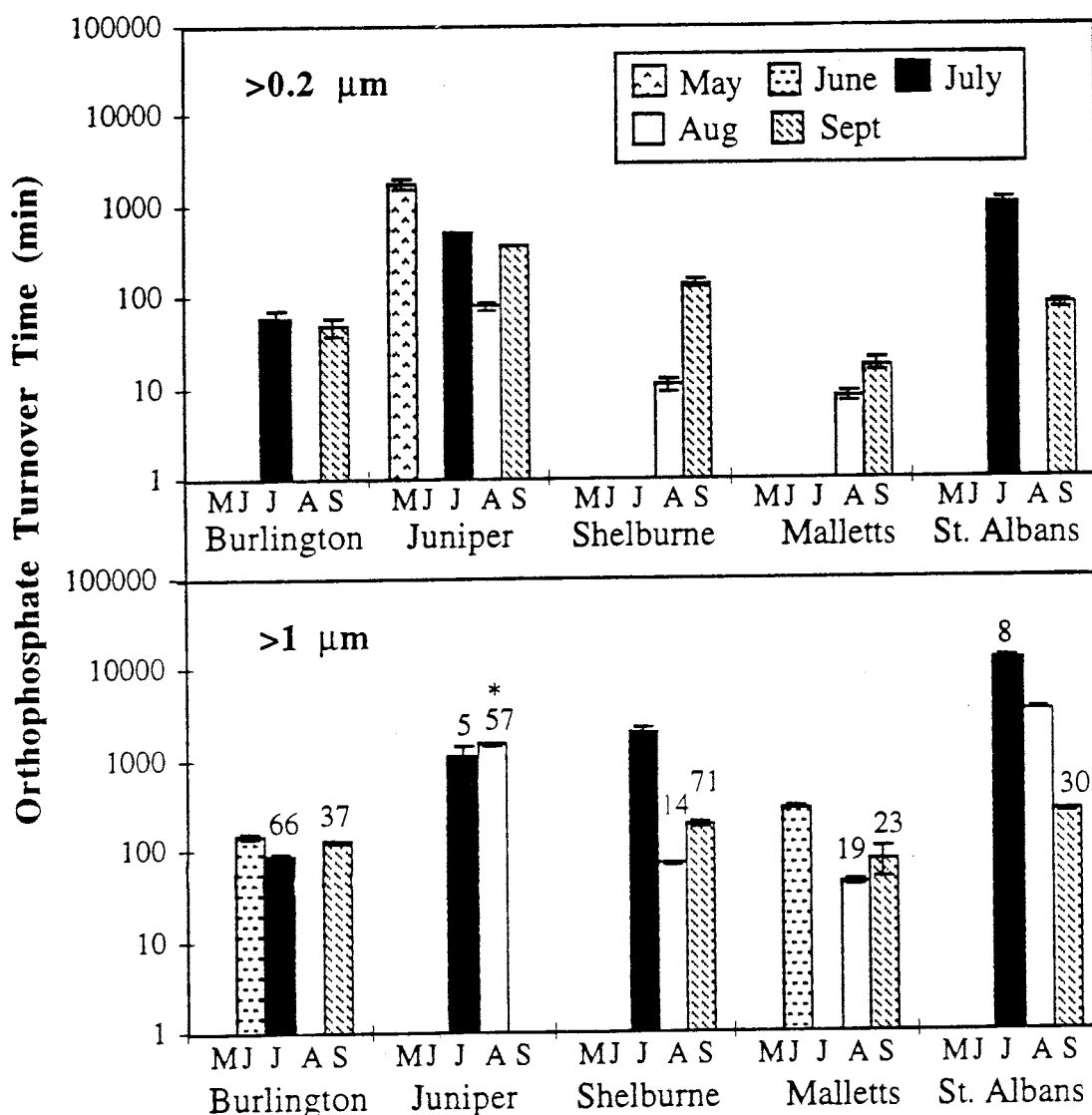


Figure 6. Orthophosphate turnover times at five sites in Lake Champlain during different months of the ice-free season. The upper panel shows OTTs when uptake by particles $>0.2 \mu\text{m}$ in size was assessed; the lower panel, the results when particles $0.2\text{--}1 \mu\text{m}$ were included in the "dissolved" fraction (for Juniper Island in August (*) the relevant fraction was $0.2\text{--}3 \mu\text{m}$, as a $3 \mu\text{m}$ filter was used). Column heights indicate mean values (no column indicates no data for the site); bars standard error. The numbers above the columns in the lower panel report the percentage of total uptake due to particles $>1 \mu\text{m}$ in size ($>3 \mu\text{m}$ for Juniper Island, August). Sampling dates were: Burlington Harbor: 1 June, 12 July, and 19 Sept. 95; Juniper Island: 11 Aug. 92, 15 Sept. 94, 15 May and 31 July 95; Malletts Bay: 29 June, 10 Aug., and 19 Sept. 95; Shelburne Bay: 10 July, 8 Aug., and 26 Sept. 95; St. Albans Bay: 10 July, 28 July (plotted as August), and 19 Sept. 95.

September. The pattern is different in St. Albans Bay, which mixes throughout the ice-free season. Here OTT continues to decrease between mid-summer and fall.

Estimation of OTT using 1 μm pore-size filters rather than 0.2 μm filters resulted in substantially longer estimates. Comparison of the uptake rate constants for the two filtration series indicated that particles $>1 \mu\text{m}$ in size accounted for from 8-71% of phosphate flux to particles. These larger particles presumably were phytoplankton, while the smaller particles were mostly bacteria.

Alkaline Phosphatase Activity

Alkaline phosphatase activity (APA) was measured only during the latter part of the summer of 1995. Three of the four sites examined in August (Burlington Harbor, Shelburne Bay, and Juniper Island, but not St. Albans Bay) had an APA greater than the $0.2 \text{ nmol } \mu\text{g chl } a \cdot \text{min}^{-1}$ threshold believed to be indicative of serious P deficiency (Fig. 7). By contrast, only constitutive levels of APA were measured during the September survey.

DISCUSSION

Nutrient Limitation in Lake Champlain

The principal question that we sought to answer through our research was whether the phytoplankton of Lake Champlain are strictly P limited as current lake management policies assume. Neither our enrichment experiments nor the assays for phytoplankton P status provided evidence for this supposition. Were P the only factor controlling phytoplankton biomass, alkaline phosphatase activity in the lake would have been consistently greater than constitutive levels ($>> 0.05 \text{ nmol PO}_4 \mu\text{g chl } a \cdot \text{min}^{-1}$) and orthophosphate turnover times would have been short ($<< 60 \text{ min}$). Instead, substantial and widespread APA (all sites but St. Albans Bay) was detected in August, but only low-level (constitutive) activity in September. Orthophosphate turnover times 8-62 min in duration were measured at three sites in mid- to late summer, but were longer in early summer and fall, and at two sites, including

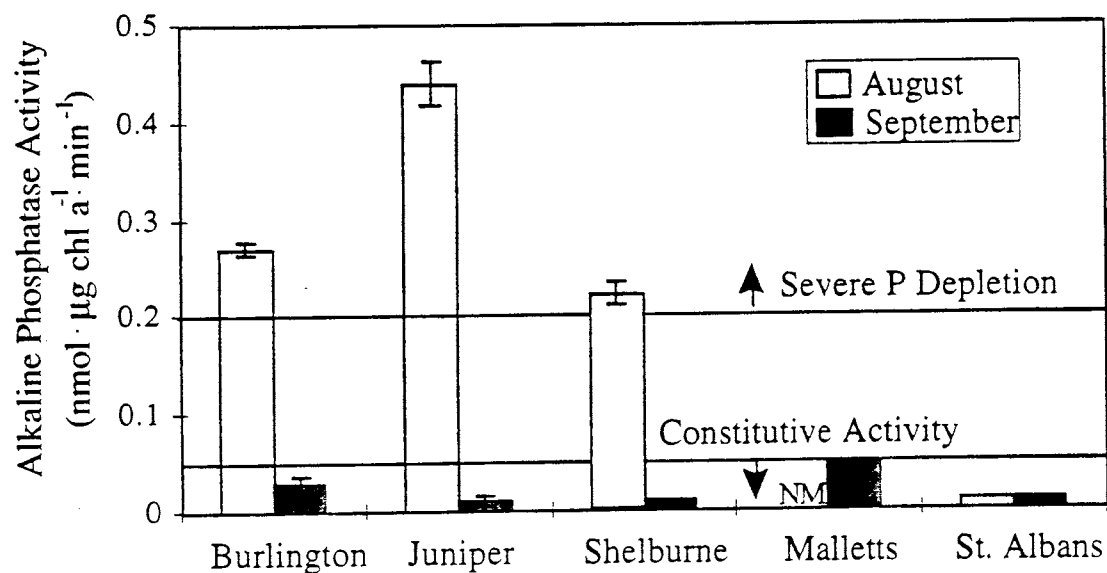


Figure 7. Chlorophyll-*a* specific alkaline phosphatase activity at five sites in Lake Champlain during the months of August and September 1995. The horizontal lines indicate critical thresholds between P sufficiency, mild P deficiency and severe P deficiency suggested by the literature. NM= not measured. Sampling dates were: Burlington Harbor: 14 Aug, 21 Sept; Juniper Island: 14 Aug, 26 Sept; Malletts Bay: 19 Sept; Shelburne Bay: 14 Aug, 26 Sept; St. Albans Bay: 31 Aug, 19 Sept.

the site of our enrichment experiments, measured OTTs never fell below 81 min. Compared with the 0.5-5 min OTTs reported for a diversity of P-deficient lakes from around the world (e.g., Peters and MacIntyre 1976; Levine and Schindler 1980; White *et al.* 1985), Lake Champlain's OTTs seem quite sluggish.

Strict P limitation is manifested in enrichment experiments as: 1) marked increases in phytoplankton biomass on the addition of P; 2) lack of response to N or Si addition; and 3) an absence of synergistic effects upon N+P or Si+P addition (i.e., the biomass in the P, N+P and Si+P carboys should be similar). While each of the four enrichment experiments revealed a somewhat different phytoplankton reaction to nutrient addition, none met all the criteria stated above. P addition never resulted in as strong a phytoplankton response as expected, given the 6-12X increase in TP concentration in the experimental carboys. In May, no response was observed, whereas in July and September, one or the other of the two response variables (chlorophyll *a* and phytoplankton biomass) did not increase enough relative to the levels in controls to be statistically different from them (17% chlorophyll *a* increase in July; 39% increase in biomass in September). Only in the June experiment did both the phytoplankton biomass and chlorophyll *a* concentrations in P treated carboys clearly exceed the levels in controls (by 5 or more fold). Silicon amendments consistently failed to stimulate phytoplankton growth, indicating that this nutrient was not growth limiting, but during one experiment (June), singular addition of N led to a small biomass increase. The most striking result of the summer experiments, however, was the consistently greater phytoplankton response to the combined addition of N and P than to P or N addition alone.

If the lake's phytoplankton are not strictly P limited, what sort of growth limitation exists? The very long (30 h) OTT that we measured in May and the failure of any combination of N, P and Si enrichment to stimulate phytoplankton growth at this time suggested a lack of nutrient limitation. These findings were not surprising as in spring, snowmelt runoff contributes a flush of nutrients to the lake, while at the same time nutrients that have accumulated at depth via decomposition and sediment return are recycled to the

surface. Deep mixing may result in light limitation, or growth may be constrained by low temperatures or grazing.

The response to enrichment that we observed in June, in which singular addition of either N or P augmented growth and combined addition of the two nutrients resulted in biomass accrual roughly equal to the sum of the biomass gains due to singular N and P additions, has been coined "reciprocal" limitation by Morris and Lewis (1988). The phenomenon is best explained by the existence of a phytoplankton community with both N- and P-limited members.

Because, in July and September, the phytoplankton in experimental carboys responded to P, but not N, addition, with increased growth, it might be concluded that the community as a whole was P limited at these times. P limitation was at best marginal, however, as the biomass increase following P addition was small (not statistically significant in July), and the OTT measured during the September experiment was well within the range of values generally indicative of P sufficiency (Lean and Pick 1981; White *et al.* 1985; Waiser and Robarts 1995). N and P had to be added in combination to elicit a major phytoplankton response. One scenario capable of explaining the observed trends is a phytoplankton community with dominants limited by P but also coping with a meager N supply. P enrichment might then stimulate growth just enough to induce N limitation. Consequently, the two nutrients would have to be added in combination to elicit a major growth response.

The observed responses to enrichment were also not too different from those expected during a phenomenon that Morris and Lewis (1988) referred to as concurrent N and P limitation. During concurrent limitation, N and P are supplied to phytoplankton at a ratio similar to the critical N:P demand ratios of the dominant species. Consequently, singular addition of P quickly brings on N limitation, while addition of N brings on P limitation. Thus, biomass accumulation is possible only when both limitation barriers are removed through N+P addition. While this sort of limitation seems like it ought to be fortuitous and

rare, it has been reported for several lakes worldwide (e.g., White and Payne 1977; Zaret *et al.* 1981; Setaro and Melack 1984; Morris and Lewis 1988).

Dissolved inorganic nutrient concentrations can provide information on the availability of nutrient to phytoplankton. While, because of possible rapid recycling, low concentrations do not necessarily imply low availability of a nutrient, seriously N-deficient phytoplankton do not allow nitrate or ammonium to accumulate in solution, nor do P-limited phytoplankton let SRP concentrations exceed detection limits (Harris 1986). At all 5 of our study sites on Lake Champlain, ammonium is generally depleted to detection limits during the growth season, but at only one of these sites (St. Albans Bay) is nitrate also depleted (Table 1). SRP generally is brought down to detection limits in Burlington Harbor and Malletts Bay, but not in St. Albans Bay or in the Main Lake. Thus both physiological indicators and inorganic nutrient concentrations hint at summertime N limitation in St. Albans Bay and at P limitation in Malletts Bay and Burlington Harbor, but provide no evidence for persistent limitation by either nutrient in the Main Lake.

Taxonomic Differences in Limitation Type

Differences among phytoplankton groups and species with regard to N, P and Si limitation were not apparent in our enrichment experiments. Diatoms were the principal phytoplankton group to accumulate biomass in response to nutrient additions in June and September, and did so regardless of whether the nutrient added was P, N or N+P. Diatoms also increased in abundance in the N+P treatment in July, but the principal group stimulated by nutrients at this time was the green algae. Cryptophytes responded positively to N+P addition, but not to singular additions of N or P, and blue-green algae responded to none of the nutrient treatments. Overall, it appears that there is considerable flexibility in which phytoplankton groups take advantage of short-term nutrient pulses.

Although we speculated that Si addition to carboys might enhance diatom growth, particularly in summer when diatoms normally diminish in numbers in Lake Champlain, this

did not happen. In their study of Lake Michigan, Schelske and Stoermer (1971) observed that Si limitation was most common at Si concentrations $<3.5 \mu\text{M}$. Concentrations this low are rare in Lake Champlain, where the upwelling associated with seiches may bring nutrients up to the surface on a regular basis. A Si concentration of just $2.8 \mu\text{M}$ was measured at the enrichment site during the week of our July experiment (Table 2), but Si addition still had no impact on either total or diatom biomass. Phosphate+Si addition produced greater biomass than P addition alone, but the stimulated species were principally green algae, rather than diatoms. Since green algae do not require appreciable amounts of Si, we believe this result to be coincidental. Either diatoms diminish in summer due to factors other than a Si shortage, or their response to Si addition requires more than four days.

Limiting Factors Other Than Nutrients

More apparent than taxon-specific reactions to carboy enrichment with nutrients were reactions of specific taxa to enclosure. In all four of our experiments, blue-green algae (small colonial species, for the most part) diminished in numbers during the 4-5 day incubation period, while cryptophytes declined in all of the carboys not supplemented with P. These cells may have been eaten by grazers in the carboys or succumbed to physiological stresses (empty and moribund cells were not enumerated). By contrast, green algae and diatoms increased in density in most control carboys. Consequently, at the end of each experiment, we generally found more differences between the phytoplankton communities in control carboys and the lake than between controls and treated carboys.

A number of factors could be responsible for the observed enclosure-induced changes in species composition: greater light exposure (carboys were incubated at 1.5-2 m depth, while natural populations mixed over 11-70 m during the experiments), elimination of mortality due to phytoplankton sinking in the carboys, decreased turbulence levels, reduced grazing pressure (due to exclusion of night-time grazers from the carboys), and a reduction in nutrient renewal processes are distinct possibilities. In any case, the observed changes

suggest that factors in addition to N, P, and Si availability affect the growth kinetics of portions of the phytoplankton community. It is even possible that the nutrient limitations that we observed in carboys reflected potential, rather than realized, limitations. For example, light-limited or grazer-limited phytoplankton relieved of these limitations by incubation conditions might develop N or P limitation, even though these limitations were not present in the lake.

The role of light in regulating phytoplankton growth in Lake Champlain, and other large lakes, is clearly a topic deserving more attention. During several months of the year (October-January; April-May), Lake Champlain mixes to the bottom (as deep as 122 m), whereas, in summer, the depth of the photic zone (7-12 m in the Main Lake) is similar to the epilimnetic depth (8-13 m) (Table 2; NY DEC, unpublished data). Thus, adaptations to low (or variable) light intensity seem necessary and could potentially influence phytoplankton composition and limit biomass. Light availability affects not only photosynthesis, but also nitrogen fixation and inorganic nutrient uptake (via its impact on cyclic phosphorylation) (Reynolds 1984). Competition studies in the laboratory have shown that niche separation of phytoplankton species occurs along both light and nutrient gradients: at low light intensities, some species may be light and others nutrient limited (Rhee and Gotham 1981).

Evidence for or against light limitation in Lake Champlain might be gained by comparing the phytoplankton biomass attained in our study's well-lit control carboys with the levels in the lake during carboy filling. Appreciable increases in phytoplankton abundance and chlorophyll *a* in carboys would be expected under light limitation. During the May and September experiments, small increases in phytoplankton biomass were observed in the control carboys, but these increases were not matched by equal increases in chlorophyll *a* (Fig. 5). In July, neither phytoplankton biomass nor chlorophyll levels changed substantially in control carboys, whereas in June, both stocks rapidly declined. We conclude that the experiments provided no substantiating evidence for light limitation in Lake Champlain, but

we still do not dismiss the possibility, especially given that the observed effects, while small, occurred at those times when the mixed layer was deepest.

Comparison of Lake Champlain with Other Lakes

The nutrient limitation patterns found in Lake Champlain are not unusual. There have been many reports of non-nutrient limited phytoplankton growth during periods of lake mixing (Reynolds 1984). Summer conditions are of more interest to managers, however, because this is when public use of lakes is greatest. Reviewing the extensive literature on in-situ nutrient enrichment assays conducted in North American lakes in summer, Elser *et al.* (1990) found that 86% of all studies reported a statistically significant increase in phytoplankton biomass or chlorophyll *a* when N and P were added simultaneously to experimental systems. By contrast, significant increases in these parameters were noted after singular amendments with P or N in just 47% and 40% of the studies, respectively. Nitrogen+P addition yielded substantially more biomass than the highest response to singular amendments of the two nutrients in 61% of the cases. The authors concluded that N and P limitation are almost equally common in lakes and that, for many lakes, there is an apparent balance between the two limitation types. They then speculated that "the mixed physiological requirements of a multi-species assemblage may closely equilibrate with the N and P supply regime of a given lake". Similar arguments for phytoplankton adjustment to nutrient conditions and hence "balanced" phytoplankton growth (i.e., growth without nutrient limitation due to balanced nutrient use and regeneration) in lakes and the ocean have been made by Goldman (1980) and Harris (1986). By contrast, Schindler (1977) and Tilman *et al.* (1982) have argued that selection for N fixers and non-diatoms at low N:P and Si:P ratios will drive systems towards P limitation.

Recently, Guildford *et al.* (1994) suggested that the phytoplankton in large lakes (>2000 ha in area) are generally less nutrient deficient and faster growing than those in smaller lakes, the reason being that their deeper and more energetic mixed layers allow for

more efficient nutrient recycling (Fee *et al.* 1994). It is difficult to assess the role of size in promoting P and N sufficiency in Lake Champlain as nutrient status indicators have only been assessed in one nearby lake, Lake Memphremagog, 65 km to the east. However, this lake does have OTTs (9-26 min; Peters 1979) lower than those measured in Lake Champlain (Main Lake). Also compatible with the large-lake hypothesis was our finding that Lake Champlain embayments display more symptoms of P (or N) deficiency than does the Main Lake. These embayments are all partially isolated from the main body of the lake and hence function to some extent like smaller lakes.

Comparison of OTTs in Lake Champlain with measurements made for the Great Lakes suggest that Lake Champlain's phytoplankton may be less prone to P deficiency than phytoplankton in most of the Great Lakes. In Lakes Superior, Michigan, Huron and Erie, OTTs measured in summer range between 14-40, 2-79, 7-26 and 13-120 min, respectively (Nalewajko *et al.* 1981; Lean and Pick 1981; Tarapchak and Moll 1990; Lehman, Univ. Mich., pers. comm.), while the shortest OTT that we measured for Lake Champlain (main basin) was 80 min. Lake Ontario, the Great Lake closest to Lake Champlain, has OTTs most similar to those found in Lake Champlain: 12 min to 20 h in summer, and 100-600 h during lake mixing (Lean *et al.* 1987). There are few published data on APA in the Great Lakes. In Lake Ontario, a seasonal pattern similar to what we observed in Lake Champlain as been reported: levels indicative of serious P deficiency ($0.3-0.5 \text{ nmol P} \cdot \mu\text{g chl } a^{-1} \cdot \text{min}^{-1}$) in mid and late summer, followed by levels typical of P sufficiency or mild deficiency ($0.05-0.10 \text{ nmol P} \cdot \mu\text{g chl } a^{-1} \cdot \text{min}^{-1}$) in fall (Pick 1987, APA normalized to chlorophyll *a* using data from Lean *et al.* 1987). On the other hand, ultraoligotrophic Lake Superior has been found to support high APA throughout the growth season (1990-91 mean, $1.1 \text{ nmol P} \cdot \mu\text{g chl } a^{-1} \cdot \text{min}^{-1}$; Guildford *et al.* 1994). That Lake Champlain appears less P deficient than lakes several times its size seemingly contradicts the large lake hypothesis. However, such a comparison is unfair because the Great Lakes lie in basins dominated by crystalline rocks while Lake Champlain's basin contains substantial amounts of dolomite. Thus the external P

loading to Lake Champlain ($0.57 \text{ g} \cdot \text{m}^{-2} \cdot \text{y}^{-1}$; Smeltzer and Quinn 1996) is greater than that currently received by any of the Great Lakes ($0.04\text{-}0.35 \text{ g} \cdot \text{m}^{-2} \cdot \text{y}^{-1}$; Coburn *et al.* 1990). In addition, Lake Champlain has an unusually active seiche for a lake of its size (T. Manley, Middlebury College, pers. comm.).

The widespread belief that P limitation is nearly universal in lakes has partly grown out of the convincing nature of whole lake eutrophication studies (e.g., Schindler *et al.* 1978), but probably is even more attributable to correlation analysis. No less than 60 analyses of regional and global data sets have shown a statistically significant correlation between chlorophyll *a* and TP concentration (Peters 1986). The consistency of this finding argues strongly for a major role for P in constraining algal biomass in lakes. Nevertheless, it does not preclude a role for other factors as well. Scatter around the chlorophyll *a*-TP regression line is large (at a given TP concentration, chlorophyll *a* concentrations may vary over two orders of magnitude) and different studies have reported somewhat different regressions coefficients. Furthermore, chlorophyll *a* correlates with TN as well as with TP, albeit at a lower level of significance (Sakamoto 1966; Smith 1982). Recently, it has been shown that division of lake data sets into subsets on the basis of TN:TP ratio (Smith 1982; Prairie *et al.* 1989), planktivorous fish density (Proulx *et al.* 1996), density of large *Daphnia* (Mazumder 1994), or mixing regime (Proulx *et al.* 1996) results in chlorophyll *a*-TP relationships with greater r^2 values. The separate regressions yield distinct regression slopes and intercepts, as would be expected if the factors used to sort the data sets play a role in determining biomass accrual.

That mortality factors such as grazing and sinking as well as nutritional factors should influence phytobiomass is readily apparent from the growth equations fundamental to population ecology. For a given population,

$$N_t = N_0 e^{rt}, \text{ and } r = b - d, \quad (1)$$

where N_t and N_0 are abundance (or biomass) at times t and 0, respectively, t is time, b is rate of increase in numbers (or biomass) due to reproduction (and also due to cell enlargement in

the case of biomass gain) and d is loss rate. (In reality, r usually fluctuates, so this equation applies to short time periods; longer periods require an integration.) Obviously, both population growth rate (r) and standing stock (N_t) are functions of the *balance* between b and d . Phosphorus exerts its influence through its impact on b . For this element to totally dictate standing stocks then, not only must all resources other than P be readily available, but d must be negligible (or linked to b in such a way that r is always proportional to b). These situations seem unlikely in natural systems.

Recommendations for the Identification of Nutrient Limitations

Because the phytoplankton communities in our control carboys were altered over the course of each of our four experiments, we suggest that investigators try to avoid enrichment experiments in carboys or other vessels that do not allow for natural mixing of phytoplankton over the depth of the epilimnion, for zooplankton migration, and natural sinking dynamics. Studies in mesocosms may be preferable, although they too have problems related to their unusual physical regime, exclusion of watershed nutrient inputs, and sensitivity to variations in founder communities (Gearing 1989). In addition, mesocosms are expensive and difficult to maintain in large lakes with deep mixed layers, currents, and heavy wave action.

Measurement of physiological and compositional indicators of nutrient status may be a more practical and affordable approach to assessing phytoplankton nutrient limitation in large lakes. Tests specific to algae are required, however, and our studies suggest that the indices that we used did not meet this criterion. A substantial role for bacteria in orthophosphate uptake dynamics was indicated by comparison of k values for uptake by particles larger than $1\ \mu\text{m}$ (presumably phytoplankton) with those for particles $>0.2\ \mu\text{m}$ (phytoplankton plus bacteria): 29-92% of orthophosphate uptake was attributable to particles in the $0.2\text{-}1\ \mu\text{m}$ size range. We measured APA only on whole water samples, and thus cannot directly address the issue of bacterial interference. However, APA production by P-

deficient bacteria is well documented (Chróst 1990). Thus, our estimates of chlorophyll-*a* specific APA are probably overestimates.

Bacteria have not been given due attention in setting up criteria for assessing the nutrient status of natural phytoplankton assemblages because, until recently, researchers assumed that bacteria obtained all the inorganic nutrient they needed from the organic matter they degraded, and thus were chronically C-limited (Cole 1982). Recent studies of the response of natural bacterial populations to nutrient enhancement, however, have shown that growth limitation by N, and more so by P, is not uncommon (e.g., Gächter *et al.* 1988; Vadstein *et al.* 1988; Toolan *et al.* 1991; Morris and Lewis 1992). At low nutrient concentrations, bacteria are superior competitors relative to algae both for orthophosphate (e.g., Currie and Kalff 1984; Cembella *et al.* 1984) and dissolved organic P (Bentzen and Taylor 1991). Therefore, studies that use OTT and APA as indicators of phytoplankton P status should include corrections for the activity of bacteria. While it might be argued that bacterial depletion of P will almost certainly bring on phytoplankton growth limitation by P, this relationship has not yet been verified. With the bacterial component removed from the indicators, the criteria used here for distinguishing between P deficiency and sufficiency may change. We strongly recommend that limnologists search for physiological indicators of P and N status that more clearly distinguish between phytoplankton and bacterial activities or conditions than do APA and OTT. Lean and Pick (1981) have suggested use of the indicator $P_{opt} : V_{max}$ (where P_{opt} is photosynthetic rate at optimal illumination and V_{max} is maximum phosphate uptake velocity). While P_{opt} is a parameter specific to phytoplankton, V_{max} , like OTT, includes bacterial activity. Lean and Pick have argued for algal control of V_{max} on the basis of biomass dominance by the algae.

Physiological assays of nutrient condition also may be criticized on the basis of their short time frame. The conditions revealed sometimes may be transient and thus not translate into significant impacts on growth (Vincent 1981). For example, while the onset of APA generally signals depletion of dissolved inorganic P supplies, the phosphatases produced may

(or may not) access enough organic P to circumvent P-limited growth. The most prudent approach to assessing nutrient limitation in lakes may be a combination of enrichment studies in mesocosms (smaller systems when necessary) and physiological and compositional assays.

While epilimnetic TN:TP ratios have been widely used to assess the likelihood of P vs. N limitation in lakes, we do not recommend use of this tool, except in conjunction with other means of assessment. During our enrichment experiments, TN:TP ratios were always in a range indicative of severe P limitation (68:1-139:1) even when P sufficiency was suggested by physiological indicators and mixed P and N limitation by growth bioassays. DIN:TP ratios (28-35:1) were much lower than TN:TP ratios, but still suggested P limitation. Smith's (1983) observation that N influences biomass up to an TN:TP ratio of 77:1 might explain our finding of mixed N and P limitation in June. However, the principal problem with using TN:TP and DIN:TP ratios as indicators of N and P status may be the fact that they can deviate substantially from the ratios at which nutrients are recycled and used. It is the flux of N and P made available to phytoplankton that affects growth rates, not the standing stocks (Harris 1986).

Implications of Our Results for Lake Champlain Management

Although we argue here that N shares a role with P in regulating phytoplankton biomass in Lake Champlain, we believe that the best strategy for reducing Lake Champlain's eutrophication problem is P control. P is much more easily removed from wastewaters than N, and best management practices aimed at controlling nonpoint sources of P generally reduce N inputs to lakes as well. Furthermore, reduction in P loading can induce P limitation when this condition is not already present in a lake. Research and monitoring programs, on the other hand, must focus on N as well as P. Eutrophication models that ignore N may lead to predictions about the response of phytoplankton to P reductions that will not immediately materialize. More seriously, the synergistic effects of N and P found in this study (and also in the EPA study) suggest that increased N loading in the absence of P cutbacks could in

itself lead to increased lake productivity. Axler *et al.* (1994) have provided evidence that algal growth in northern Minnesota lakes has been stimulated by the N entering lakes in acid precipitation. While agriculture accounts for most of the N rich runoff currently entering Lake Champlain, nitric acid deposition is greater in New England than in northern Minnesota and is increasing (NADP 1996).

Conclusions

Much more about environmental controls on growth must be known to truly understand and accurately model phytoplankton dynamics in Lake Champlain. For many years, mainstream limnology has tended to simplify reality by modelling eutrophication as a process driven solely by P supply. This practice assumes a uniformity among phytoplankton species with regard to nutrient requirements and nutrient acquisition abilities exists has not been borne out by autecological studies in the laboratory. The extent to which species replacements drive nutrient demand ratios toward a narrower range compatible with general nutrient sufficiency (or P limitation) is unknown. In the current study, we have shown that P is an important factor in constraining phytoplankton growth in Lake Champlain, but apparently does not act alone in this role. Alternating periods of P deficiency and sufficiency were suggested by the OTT and APA values that we measured for the lake, while our enrichment experiments suggested that N is scarce enough in the lake that P addition sometimes yields very little increase in phytobiomass before N limitation is induced. That factors other than P and N (perhaps light, sinking, or grazing) may also influence phytobiomass was hinted at by the species successions we observed in control carboys. This apparent Pandora's box of potential limitations in Lake Champlain is consistent with resource competition theory (Tilman *et al.* 1982), as well as with the growth equations of population ecology (Eq. 1), but not with eutrophication theory.

No one can argue that the premise of general P limitation in lakes and the empirical models developed to predict phytoplankton biomass (chlorophyll *a*) from TP concentration

or P loading haven't benefitted eutrophication management. They have encouraged P cutbacks that have led to improved water quality in many lakes. On the other hand, the practice of treating phytoplankton communities as single entities controllable by only a single factor has had a dampening effect on phytoplankton research. Thus it is time that we allow resource competition theory, which relies on species diversity in nutrient requirements, uptake abilities and limitations to explain species diversity and succession, and "top-down" theory, which emphasizes phytoplankton species differences in vulnerability to grazers to explain the same trends, to achieve a better mesh with our concepts about eutrophication.

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REFERENCES

- Axler, R.P., Rose, C., and Tikkanen, C.A. 1994. Phytoplankton nutrient deficiency as related to atmospheric nitrogen deposition in northern Minnesota acid-sensitive lakes. *Can. J. Fish. Aquat. Sci.* 51: 1281-1296.
- Bentzen, E. and W.D. Taylor. 1991. Estimating organic P utilization by freshwater plankton using [32P]ATP. *J. Plankton Res.* 13: 1223-1238
- Cembella, A.D., N.J. Anita, and P.J. Harrison. 1984. The utilization of inorganic and organic phosphorus compounds as nutrients by eukaryotic microalgae: A multidisciplinary perspective: Part I. *CRC Crit. Rev. Microbiol.* 10: 317-391.
- Chróst, R.J. 1990. *Microbial ectoenzymes in aquatic environments*. New York: Springer-Verlag.
- Cole, J.J. 1982. Interactions between bacteria and algae in aquatic ecosystems. *Ann. Rev. Ecol. Syst.* 13: 291-314.
- Colborn, T.E., A. Davidson, S.N. Green, R.A. Hodge, C.I. Jackson, and R.A. Liroff. 1990. *Great Lakes. Great Legacy?*, Washington D.C. and Ottawa, Ont.: Conservation Foundation and Institute for Research on Public Policy
- Cullen, J.J. 1991. Hypotheses to explain high-nutrient conditions in the open sea. *Limnol. Oceanogr.* 36: 1578-1599.
- Currie, D.J., and J. Kalff. 1984. A comparison of the abilities of freshwater algae and bacteria to acquire and retain phosphorus. *Limnol. Oceanogr.* 29: 298-310.
- Downing, J.A., and McCauley, E. 1992. The nitrogen:phosphorus relationship in lakes. *Limnol. Oceanogr.* 37: 936-945.
- Elser, J.J., Marzolf, E.R., and Goldman, C.R. 1990. Phosphorus and nitrogen limitation of phytoplankton growth in the freshwaters of North America: A review and critique of experimental enrichments. *Can. J. Fish. Aquat. Sci.* 47: 1468-1477.

- Fee, E.J., R.E. Hecky, G.W. Regehr, L.L. Hendzel, and P. Wilkinson. 1994. Effects of lake size on nutrient availability in the mixed layer during summer stratification. *Can. J. Fish. Aquat. Sci.* 51: 2756-2768.
- Gächter, R., Meyer, J.S., and Mares, A. 1988. Contribution of bacteria to release and fixation of phosphorus in lake sediments. *Limnol. Oceanogr.* 33: 1542-1558.
- Gage, M.A., and Gorham, E. 1985. Alkaline phosphatase activity and cellular phosphorus as an index of the phosphorus status of phytoplankton in Minnesota lakes. *Freshwater Biol.* 15: 227-233.
- Gearing, J.N. 1989. The role of aquatic microcosms in ecotoxicologic research as illustrated by large marine systems. In *Ecotoxicology: Problems and Approaches*, eds. S.A. Levine, M.A. Harwell, J.R. Kelly and K.D. Kimball, New York: Springer-Verlag, pp. 411-472.
- Goldman, J.C. 1980. Physiological processes, nutrient availability and the concept of relative growth rate in marine phytoplankton ecology, In *Primary Production in the Sea*, ed. P. Falkowski, New York: Plenum, pp. 179-194.
- Gruendling, G.K. 1974. Nutrients, phytoplankton, and limiting factors in Lake Champlain, New York and Vermont., *Conference on Water Research on Lake Champlain*, Vermont Water Resources Research Center, pp. 39-49.
- Guildford, S.J., L.L. Hendzel, H.J. Kling, E.J. Fee, G.G.C. Robinson, R.E. Hecky, and S.E.M. Kasian. 1994. Effects of lake size on phytoplankton nutrient status. *Can. J. Fish. Aquat. Sci.* 51: 2769-2783.
- Harris, G.P. 1986. *Phytoplankton Ecology. Structure, Function and Fluctuation*. London: Chapman and Hall.
- Howarth, R.W. 1988. Nutrient limitation of net primary production in marine ecosystems. *Ann. Rev. Ecol. Syst.* 19: 89-110.
- Jansson, M., Olsson, H., and Pettersson, K. 1988. Phosphatases: origin, characteristics and function in lakes. *Hydrobiol.* 170: 157-175.

- Lean, D.R.S., and Pick, F.R. 1981. Photosynthetic response of lake plankton to nutrient enrichment: a test for nutrient limitation. *Limnol. Oceanogr.* 26: 1001-1019.
- Levine, S.N., and Schindler, D.W. 1980. Radiochemical analysis of orthophosphate concentrations and seasonal changes in the flux of orthophosphate to seston in two Canadian Shield lakes. *Can. J. Fish. Aquat. Sci.* 37: 479-487.
- Levine, S.N., and Schindler, D.W. 1992. Modification of the N:P ratio in lakes by in situ processes. *Limnol. Oceanogr.* 37: 917-935.
- Lorenzen, C.J. 1967. Determination of chlorophyll and pheopigments: Spectrophotometric equations. *Limnol. Oceanogr.* 12: 343-346.
- McIntosh, A., E. Brown., A. Duchovnay, A. Shambaugh, and A. Williams. 1993. *1992 Lake Champlain Biomonitoring Program*. Burlington, VT: University of Vermont. Vermont Water Resources and Lake Studies Center.
- Morris, D.P., and Lewis, W.M., Jr. 1988. Phytoplankton nutrient limitation in Colorado mountain lakes. *Freshwater Biol.* 20: 315-327.
- Morris, D.P., and Lewis, W.M.J. 1992. Nutrient limitation of bacterioplankton growth in Lake Dillon, Colorado. *Limnol. Oceanogr.* 37: 1179-1192.
- Mamumder, A. 1994. Phosphorus-chlorophyll relationships under contrasting herbivore and thermal stratification: predictions and patterns. *Can. J. Fish. Aquat. Sci.* 51: 390-400.
- Myer, G.E. and G.K. Gruendling. 1979. *Limnology of Lake Champlain*. Burlington, VT: New England River Basin Commission,.
- Nalewajko, C., K. Lee, and H. Shear. 1981. Phosphorus kinetics in Lake Superior: light intensity and phosphate uptake in algae. *Can. J. Fish. Aquat. Sci.* 38: 224-232.
- National Atmospheric Deposition Program/National Trends Network. 1996. *NADP/NTN Wet Deposition in the United States 1995*. Fort Collins, CO.: Natural Resource Ecology Laboratory, Colorado State Univ.

- Peters, R.H. 1975. Orthophosphate turnover in central European lakes. *Mem. Ist. Ital. Idrobiol.* 32:
- Peters, R.H. 1979. Concentrations and kinetics of phosphorus fractions along the trophic gradient of Lake Memphremagog. *J. Fish. Res. Board Can.* 36: 970-979.
- Peters, R.H. 1986. The role of prediction in limnology. *Limnol. Oceanogr.* 31: 1160-1166.
- Peters, R.H., and MacIntyre, S. 1976. Orthophosphate turnover in East African lakes. *Oecologia* 25: 313-319.
- Pettersson, K. 1980. Alkaline phosphatase activity and algal surplus phosphorus as phosphorus-deficiency indicators in Lake Erken. *Arch. Hydrobiol.* 89: 57-87.
- Pick, F. R. 1987. Interpretations of alkaline phosphatase activity in Lake Ontario. *Can. J. Fish. Aquat. Sci.* 44: 2087-2094.
- Prairie, Y.T., C. M. Duarte, and J. Kalff. 1989. Unifying nutrient-chlorophyll relationships in lakes. *Can. J. Fish. Aquat. Sci.* 46: 1176-1182.
- Proulx, M., F.R. Pick, A. Mazumder, P.B. Hamilton, and D.R.S. Lean. 1996. Effects of nutrients and planktivorous fish on the phytoplankton of shallow and deep aquatic systems. *Ecology* 77: 1556-1572.
- Redfield, A.C. 1958. The biological control of chemical factors in the environment. *Amer. Sci.* 46: 205-222.
- Reynolds, C.S. 1984. *The Ecology of Freshwater Phytoplankton*. Cambridge: Cambridge University Press.
- Rhee, G.-Y. 1978. Effects of N:P atomic ratios and nitrate limitation on algal growth, cell composition, and nitrate uptake. *Limnol. Oceanogr.* 23: 10-25.
- Rhee, G.-Y., and Gotham, I.G. 1980. Optimum N:P ratios and coexistence of planktonic algae. *J. Phycol.* 16: 486-489.
- Rhee, G.-Y., and Gotham, I.G. 1981. The effect of environmental factors on phytoplankton growth: Light and interactions of light with nitrate limitation. *Limnol. Oceanogr.* 26: 649-659.

- Rigler, F.H. 1964. The phosphorus fractions and the turnover time of inorganic phosphorus in different types of lakes. *Limnol. Oceanogr.* 9: 511-518.
- Sakamoto, M. 1966. Primary production by phytoplankton community in some Japanese lakes and its dependence on lake depth. *Arch. Hydrobiol.* 62: 1-28.
- Sartory, D.P., and Grobbelaar, J.U. 1984. Extraction of chlorophyll a from freshwater phytoplankton for spectrophotometric analysis. *Hydrobiol.* 114: 177-187.
- Schindler, D.W. 1977. The evolution of phosphorus limitation in lakes. *Science* 195: 260-262.
- Schindler, D.W., E.J. Fee, T. Ruscynski. 1978. Phosphorus input and its consequences for phytoplankton standing crop in the Experimental Lakes Area and similar lakes. *J. Fish. Res. Bd. Can.* 35: 190-196.
- Schleske, C.L., and Stoermer, E.F. 1971. Phosphorus, silica and eutrophication of Lake Michigan. *Spec. Sympos. Amer. Soc. Limnol. Oceanogr.* 1: 157-171.
- Setaro, F.V., and Melack, J.M. 1984. Responses of phytoplankton to experimental nutrient enrichment in an Amazon floodplain lake. *Limnol. Oceanogr.* 29: 972-984.
- Smeltzer, E., and S. Quinn. 1996. A phosphorus budget, model, and load reduction strategy for Lake Champlain. *J. Lake Reservoir Managem.* 12: 381-393.
- Smith, R.E., and Kalff, J. 1981. The effect of phosphorus limitation on algal growth rates: Evidence from alkaline phosphatase. *Can. J. Fish. Aquat. Sci.* 38: 1421-1427.
- Smith, V.H. 1982. The nitrogen and phosphorus dependence of algal biomass in lakes: an empirical and theoretical analysis. *Limnol. Oceanogr.* 27: 1101-1112.
- Smith, V.H. 1983. Low nitrogen: phosphorus ratios favor dominance by blue-green algae in lake phytoplankton. *Science* 221: 669-671.
- Sokal, R.R., and Rohlf, F.J. 1981. *Biometry: The Principles and Practice of Statistics in Biological Research*. San Francisco: W.H. Freeman and Company.
- Tarapchak, S.J. and R.A. Moll. 1990. Phosphorus sources for phytoplankton and bacteria in Lake Michigan. *J. Plankton Res.* 12: 743-758.

- Terry, K.L. 1980. Nitrogen and phosphorus requirements of *Pavlova lutheri* in continuous culture. *Bot. Marina* 13: 757-764.
- Tilman, D., Kilham, S.S., and Kilham, P. 1982. Phytoplankton community ecology: The role of limiting nutrients. *Ann. Rev. Ecol. Syst.* 13: 349-372.
- Toolan, T., Wehr, J.D., and Findlay, S. 1991. Inorganic phosphorus stimulation of bacterioplankton production in a meso-eutrophic lake. *Appl. and Env. Microbiol.* 57: 2074-2078.
- U.S. EPA. 1974. *EPA National Eutrophication Survey. Report on Lake Champlain, New York and Vermont, EPA regions I and II.* Corvallis, OR: Pacific Northwest Environmental Research Lab.
- Vadstein, O., Jensen, O., Olsen, Y., and Reinertsen, H. 1988. Growth and phosphorus status of limnetic phytoplankton and bacteria. *Limnol. Oceanogr.* 33: 489-503.
- VT DEC and NY State DEC. 1994. *A Phosphorus Budget, Model, and Load Reduction Strategy for Lake Champlain. Lake Champlain Diagnostic-Feasibility Study Final Report.*
- Vincent, W.F. 1981. Rapid assays for nutrient demand by the plankton. I. Nitrogen, II. Phosphorus. *J. Plankton Res.* 3: 685-710.
- Waiser, M.J., and Robarts, R. 1995. Microbial nutrient limitation in prairie saline lakes with high sulfate concentration. *Limnol. Oceanogr.* 40: 566-574.
- Walker, W.W.J. 1986. *Perspectives on Eutrophication in Lake Champlain,* VT Department of Fish and Wildlife.
- Wetzel, R.G., and Likens, G.E. 1991. *Limnological Analyses.* New York: Springer-Verlag.
- White, E., Law, K., Payne, G., and Pickmere, S. 1985. Nutrient demand and availability among planktonic communities- an attempt to assess nutrient limitation to plant growth in 12 central volcanic plateau lakes. *New Zealand J. Mar. Freshwater Res.* 19: 49-62.

- White, E., and Payne, G.W. 1977. Chlorophyll production in response to nutrient additions, by the algae in Lake Taupo water. *New Zealand J. Mar. Freshwater Res.* 11: 501-507.
- Zaret, T.M., Devol, A.H., and Dos Santos, A. 1981. Nutrient addition experiments in Lake Jacaretinga, Central Amazon Basin, Brazil. *Verh.-Int. Ver Theor. Angew. Limnol.* 21: 721-724.

SECTION IV

The Impact of Zooplankton Grazing on Phytoplankton Species Composition and Biomass in Lake Champlain (USA-Canada)

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ABSTRACT

Rates of zooplankton grazing on phytoplankton were determined for the main basin of Lake Champlain using a technique pioneered by Lehman and Sandgren (1985). Three densities each of macrograzers (cladocerans, copepodite and adult copepods) and micrograzers (rotifers and nauplii) were created in duplicate in carboys filled with lake water and supplemented with nutrients to minimize the effects of zooplankton nutrient regeneration. The carboys were incubated *in situ* for 2-3 days in May, July and September. Clearance rates for specific phytoplankton taxa were determined from the slopes of regressions of taxon growth rates on grazer biomass. Substantial variability in grazing mortality was observed among phytoplankton species, both within and between divisions. In general, medium-sized phytoplankton (5-20 μm) were more heavily grazed than much smaller or larger species. Dinoflagellates and green algae suffered greater losses to macrozooplankton grazers (6-26% of biomass per day) than did cryptophytes (1-8% $\cdot\text{day}^{-1}$), diatoms (0-7% $\cdot\text{day}^{-1}$) and blue green algae (0-6% $\cdot\text{day}^{-1}$), but were not heavily grazed by microzooplankton. In general, microzooplankton had higher specific clearance rates than macrozooplankton, but were less common in the lake so that removal rates by the two grazer size categories were similar. Overall losses to grazers amounted to 2-21% of phytoplankton biomass and 3-117% of primary productivity per day.

In a 3 X 2 factorial experiment in which grazers and nutrients (phosphorus, nitrogen and glucose) were manipulated simultaneously, nutrients consistently stimulated phytoplankton growth, while phytoplankton response to zooplankton depended on nutrient treatment. At ambient nutrient levels, phytobiomass (chlorophyll a) increased with total zooplankton (herbivore plus carnivore) density, possibly due to animal nutrient recycling, while at saturating nutrient levels, phytobiomass was inversely correlated with herbivore abundance. We conclude that both nutrients and grazers are important in structuring Lake Champlain's phytoplankton community.

Key words: Lake Champlain, zooplankton, phytoplankton, grazing, nutrients, primary productivity

INTRODUCTION

Demonstrations that planktivorous fish and zooplankton communities can be manipulated through predator addition or removal (e.g., Hrbacek et al. 1961; Brooks and Dodson 1965; Galbraith 1967; Hutchinson 1971; Stenson et al. 1978; Shapiro and Wright 1984; Mills et al. 1987; Reinertsen et al. 1990) have sparked the development of trophic cascade theory (Carpenter et al. 1985) and raised questions about the relative roles of nutrients and zooplankton in controlling phytoplankton biomass and composition. Particularly controversial have been suggestions that biomanipulation (piscivorous fish stocking) be used as a management tool for controlling algal-related problems in lakes where phosphorus control has proven ineffective or difficult (Shapiro et al. 1975, 1982; Shapiro and Wright 1984).

Limnologists have long recognized the potential for grazers to minimize phytoplankton standing stocks. The clear water phase common in early summer in many lakes has been attributed to grazing losses much in excess of reproduction (Tilzer 1984; Lampert et al. 1986; Sommer et al. 1986). That grazers also influence phytoplankton community structure has been shown in field experiments in which grazer density has been manipulated and changes in species composition have resulted (e.g., Porter 1973; Berquist and Carpenter 1986, Svensson and Stenson 1991; Proulx et al. 1986). In addition, the gut contents of zooplankton have been compared with the make-up of their food supply to demonstrate differential vulnerability to grazing (Porter 1973; Infante 1978; Berquist and Carpenter 1986). Porter (1973, 1977) has suggested that many of the morphological features of phytoplankton are adaptations to minimize grazing mortality: larger size ($>30\text{ }\mu\text{m}$), spines, and aggregation into filaments or colonies may reduce the chance of ingestion, while mucilaginous sheaths and durable coverings may enable algae to pass through zooplankton guts without digestion. Naked flagellates are believed to be highly edible, but counter grazing losses with high reproductive rates.

The zooplankton-phytoplankton relationship is complex, as it involves not only feeding interactions, but nutrient exchange. Zooplankton excretion is a major source of inorganic nitrogen and phosphorus for phytoplankton in most lakes (Larow and

McNaught 1978; Lehman 1980). Thus increases in zooplankton density often stimulate specific primary productivity (carbon fixed per unit biomass) even as they diminish phytoplankton standing stocks (Elser and MacKay 1989; Svensson and Stenson 1991; Elser 1992). Furthermore, zooplankton may play a role in determining whether the phytoplankton in a lake are nitrogen or phosphorus limited by recycling these scarce nutrients at a ratio above or below that required by phytoplankton (Elser et al. 1988; Sterner 1990; Sarnelle 1992).

Zooplankton grazing on phytoplankton has been studied extensively in the laboratory, as well as in field chambers, but usually from a bottom-up perspective, so that the repertoire of prey examined has been small. From these studies it is known that grazing rates are dependent on the taxonomy, life stage and size of grazers, on environmental conditions such as temperature and turbidity, and on food quantity and quality (e.g., Haney 1973, Bogdan and McNaught 1975, DeMott 1982, Gulati et al 1982, Bogdan and Gilbert 1987, Sterner 1989, Garnier and Mourelatos 1991, Cyr and Pace 1992, Sarnelle 1992).

Most field studies of phytoplankton response to grazing have been qualitative, depending on ANOVA to verify effects. Quantification of taxon-specific grazing rates is necessary, however, if grazing is to be incorporated into models of phytoplankton community dynamics. Two methods for estimating grazing rates on natural phytoplankton assemblages in the field have been pioneered in recent years. Both involve manipulating grazer densities in in-situ enclosures and measuring the subsequent phytoplankton growth (en masse or by taxa) over an incubation period. It is assumed that the manipulations alter grazing pressure, but not reproductive rate or other sources of mortality, such as sinking or parasitism. In one method, half of the enclosures contain grazers at ambient levels and the other half no grazers (e.g., Elser and Goldman 1991; Elser 1992). Grazing rate is estimated as the difference between phytoplankton growth rates in the two sets:

$$1. \quad g_z - g_0 = (r - m_g - m_a) - (r - 0 - m_a) = m_g,$$

where g_z and g_0 are the population growth rates in the presence and absence of zooplankton, r is reproductive rate, m_g is grazing mortality and m_a is mortality due to other causes.

A drawback of this method is that 100% removal of grazers is difficult to achieve. Consequently, Lehman and Sandgren (1985), developed a method that avoids this requirement. A gradient of grazer densities (three or more) is produced in enclosures and the slope of the regression of phytoplankton growth rates in the enclosures on grazer densities is used to estimate grazer clearance rate (Fig. 1). The product of clearance rate and ambient zooplankton density (or biomass) yields an estimate of grazing rate expressed as a proportion of cells (or biomass) removed per day. While grazer manipulation has traditionally occurred through sieving and additions, techniques involved dilution of experimental systems with filtered water (which decreases encounter rates) have been developed recently as well (Landry and Hassett 1982; Elser and Frees 1995)

Both the grazer-removal and Lehman-Sandgren method require that nutrients be added to experimental systems to saturate phytoplankton uptake of limiting nutrient. If this is not done, the amount of nutrient recycled by zooplankton varies across treatments, possibly leading to different reproductive rates at different grazer levels. Early pioneers of grazing methods did not add nutrients and thus reported "net" loss (grazing - stimulated growth) rather than grazing rates. Most chose to avoid reporting rates at all, but instead grouped taxa according to whether their net response to grazers was negative, positive, or neutral (e.g., Lehman and Sandgren 1985; Berquist and Carpenter 1986). Consequently, the only extensive data set on algal-species specific grazing rates is that of Elser and Goldman (1991; Elser 1992) for three California lakes. In addition, Cyr and Pace (1992) have used the Lehman and Sandgren method with nutrient addition to estimate community-level grazing mortality (chlorophyll *a* loss) in northeastern U.S. lakes. In two of these lakes, they also examined *Cryptomonas* mortality

Obviously much more data are required before generalizations about grazing relationships can be made. Patterns must be determined across seasons and for lakes of different trophic status and physiochemical condition. Equally important, grazing studies need to be extended to

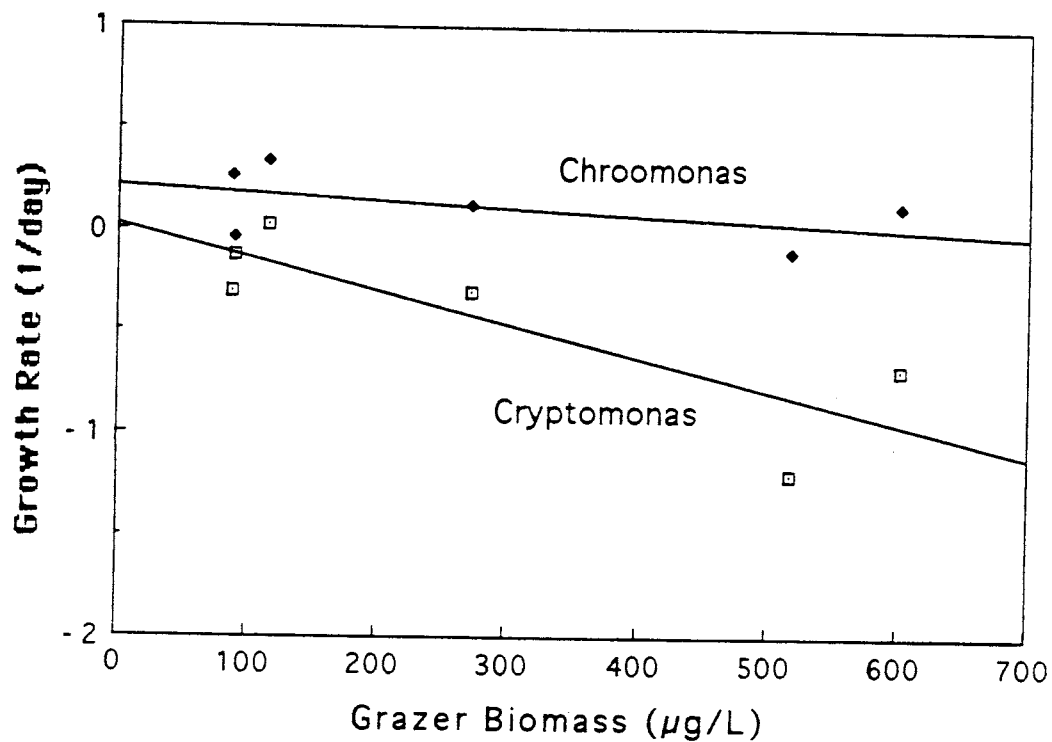


Figure 1. An example of the plots of phytoplankton growth rate on grazer biomass (or density) used to estimate grazer clearance rates. The data presented are for two species present in September 1994. Clearance rate in $\text{mL} \cdot \text{g dwt} \cdot \text{day}^{-1}$ is the slope of the plot times $1000 \text{ mL} \cdot \text{L}^{-1}$. In this case, the clearance rates were 1.6 and $0.3 \text{ mL} \cdot \text{g dwt} \cdot \text{day}^{-1}$ for *Cryptomonas* and *Chroomonas*, respectively (determined by linear regression; $r^2 = 0.69$ and 0.20).

micrograzers. The sieves and nets used to set up grazing experiments often are coarse enough to allow most rotifers and nauplii to pass. Consequently, micrograzer densities may be similar across treatments, and micrograzer activity a constant source of mortality within an experiment. Because the slope of the growth rate vs. animal density relationship is used to estimate clearance rate and micrograzing does not contribute to this slope, grazing estimates are restricted to the zooplankton component greater than the sieve size (which has ranged from 80-220 μm).

Both size efficiency and trophic cascade theory assume that large zooplankton, and especially daphnids, are more efficient and thus more important grazers than small animals (e.g., Hrbacek et al. 1961; Brooks and Dodson 1965; Shapiro and Wright 1984; Carpenter et al. 1985; Scavia et al. 1988). Thus most grazing studies have been conducted in systems where cladocerans dominate. However, Cyr and Pace (1992) found no statistical evidence within their large data set for higher grazing rates in *Daphnia*-dominated zooplankton communities than in communities dominated by smaller zooplankton. Furthermore, while laboratory studies show that larger animals have greater grazing rate on a per animal basis (e.g., DeMott 1982; Peters and Downing 1984), small animals generally graze more per unit biomass (Peters and Downing 1984; Jarvis et al. 1988; Lair 1991; Havens 1991). Since cladocerans are generally abundant only in summer and in some lakes comprise a smaller proportion of the biomass than rotifers for much or all of the year, micrograzer impacts on phytoplankton deserve more attention. Recently, Elser and Frees (1995) used a dilution method to evaluate microzooplankton grazing in Castle Lake, CA. The grazing rates they obtained were greater than those that they are previously estimated for crustacean grazing in the lake.

In this paper we describe application of the Lehman-Sandgren method (with nutrient addition) to Lake Champlain, a deep mesotrophic lake in New England. The method was applied during three seasons (spring, summer, and fall) and included separate analysis of macrozooplankton (adult and copepodite copepods and cladocerans) and microzooplankton (rotifers and nauplii) grazing. Our approach to estimating microzooplankton grazing was to first sieve macrozooplankton from the water used in our experimental systems and then manipulate

microzooplankton abundance. We report here both on grazing rates, and on methodological issues that we encountered. In addition, we present the results of an experiment in which we manipulated zooplankton biomass and nutrients simultaneously (3 X 2 factorial design) to examine the relative importance of the two factors in controlling phytoplankton biomass and species composition in Lake Champlain. The impact of our grazer and nutrient manipulations on bacteria and heterotrophic protozoa is discussed in a separate paper (Borchardt et al., Section 5). In addition, enrichment experiments conducted in concert with the grazing experiments for the purpose of identifying nutrient limitations among phytoplankton are reported on in Levine et al. (1997; Section 3).

SITE DESCRIPTION

Lake Champlain is a large (170 km long, surface area 1,130 km²), deep (maximum depth 122 m; mean depth 23 m), but narrow (maximum width, 20 km) lake straddling the border between Vermont and New York and extending a short distance into Quebec (Fig. 2). It empties north through the Richelieu River, a tributary of the St. Lawrence River, but is also connected at its southern end to the Hudson River via the Lake Champlain Canal. Numerous islands, sills, peninsulas and causeways divide the lake into partially isolated sub-basins. Our study took place in the largest of these, known as "Main Lake".

Main Lake is marginally dimictic: it stratifies in summer establishing an epilimnion with a depth of 10-12 m and in winter freezes for 0-2 months (February to April). For several months between October-June, however, it mixes to the bottom (70 m at our sampling site). Some sub-basins of Lake Champlain are eutrophic with summer blue-green blooms, but Main Lake is oligotrophic-mesotrophic (mean TP=0.4 μ M (VT DEC, unpublished data); mean chlorophyll a concentration = 5 μ g/L (NY DEC, unpublished data)). Its phytoplankton appear to be principally P limited in summer, but P stress is not severe (alkaline phosphatase activity is relatively low and orthophosphate turnover times long) and N may be co-limiting at times (Levine et al. 1997).

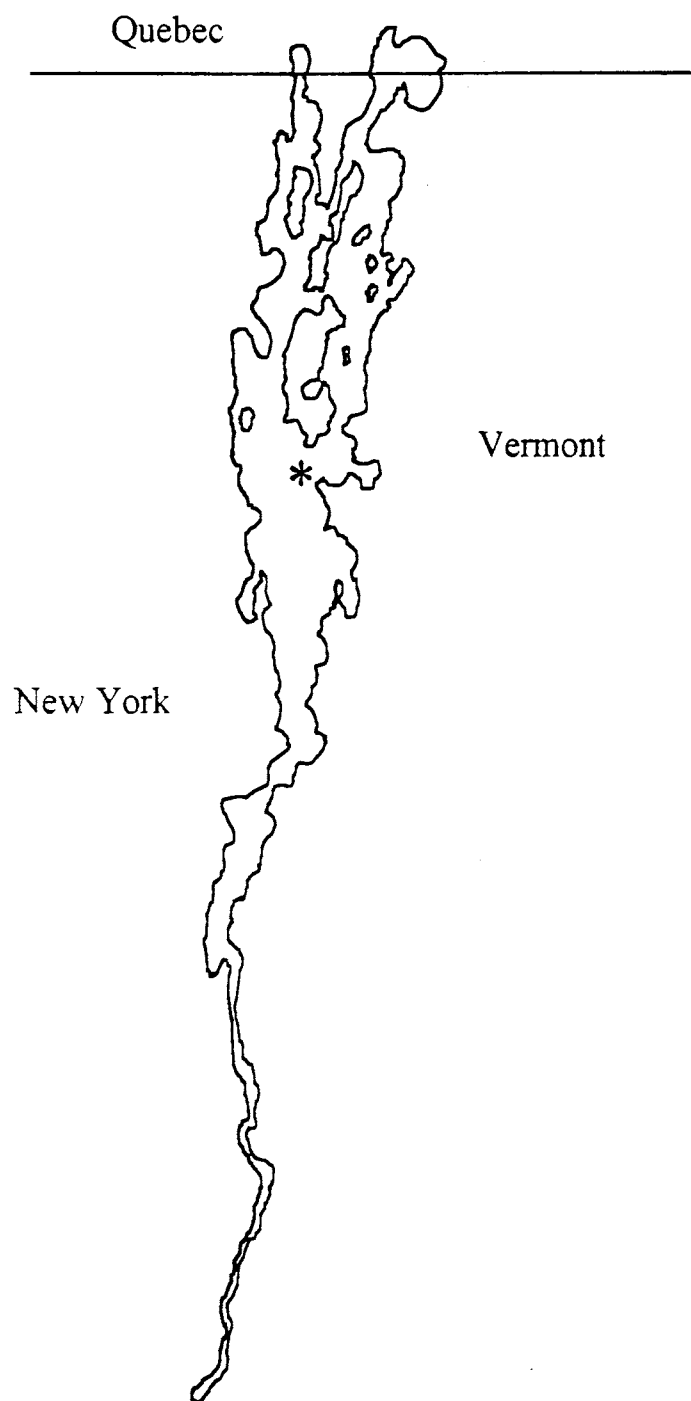


Figure 2. A map of Lake Champlain showing the study site near Juniper Island (VT DEC and NY DEC Site 19).

Main Lake's phytoplankton community typically is dominated by diatoms and cryptophytes during much of the year, but blue green algal dominance is common in late summer (McIntosh et al. 1993). Seasonal phytoplankton biomass distribution is typically bimodal, with a major peak in spring and a smaller peak in fall. The zooplankton community is a mixture of cyclopoid copepods (mostly *Mesocyclops edax* and *Diacyclops bicuspidatus thomasi*), cladocerans (mostly *Daphnia galeata mendota*, *Daphnia retrocurva*, and *Bosmina longirostris*), and rotifers (e.g., *Keratella cochlearis*, *Polyarthra major*, *Kellicottia longispina* and *Notholca* sp.) (McIntosh et al. 1993). Calanoid copepods (e.g., *Diaptomus minutus* and *Diaptomus silicis*) are comparatively scarce. In a typical year, copepods and rotifers are most abundant in spring, while cladocerans peak in summer. Over 80 species of fish are present in Lake Champlain; *Osmerus mordax dentex* (rainbow smelt) is the dominant planktivore (Myer and Gruendling 1979).

Our sampling site was at 44°27' N, 73°17' W, about 1 km northwest of Juniper Island, at the broadest expanse of the lake (Fig. 2). We chose this site because it is the joint index station of the New York and Vermont Departments of Environmental Conservation.

METHODS

Grazing Experiments

The experiments to estimate zooplankton clearance rates in Main Lake were conducted in mid-summer (July 25-27) and fall (Sept. 27-29), 1994 and again in spring (May 8-11) 1995. The design of Lehman and Sandgren (1985) was employed, with 10-liter polyethylene carboys as experimental units. Three levels of grazing pressure were created in duplicate for each of two grazer size categories, macrozooplankton (animals retained by 202 μ m mesh; adult and copepodite copepods and cladocerans) and microzooplankton (20-202 μ m; rotifers and nauplii), and phytoplankton growth response assessed over 2-3 days. Separate assessment of the impact of grazing by the two zooplankton size categories depended on total (or near total) removal of macrozooplankton from micrograzer treatments prior to micrograzer manipulation, and on maintenance of uniform microzooplankton densities across the macrozooplankton treatments (i.e.,

the sieves and nets used in this study could not concentrate microzooplankton). In addition, experimental success depended on the production of grazer gradients without alteration of either grazer or phytoplankton community structure. Alteration of grazers would mean that our goal of analyzing a particular community was not being met, while the changes in phytoplankton abundance or in the relative abundance of different species might alter grazing rates, making the relationship between phytoplankton growth rates and grazer density nonlinear.

Water and plankton for the experiment were obtained with an 8-L van Dorn bottle deployed at 1-m intervals over the depth of the epilimnion (10 m in summer, 18 m in fall) and 2 m into the metalimnion. In spring, when the lake mixed to the bottom (70 m at our site), water collection was terminated at 20 m (as in fall). This water was pooled and mixed in a 240-L polypropylene tank before dispersal to carboys or sieving. Zooplankton for our "high grazer density" treatment were obtained by hauling a 202 μm -mesh Wisconsin net (collar off; mouth diameter 0.5 m) with a mason jar attached up through the same depth interval two times and adding the captured animals to 30 L of lake water. The animals were released under the water surface to minimize air trapping under the cladoceran carapaces (which results in significant animal mortality).

The procedure for preparing the experimental treatments is illustrated in Figure 3. The "ambient macrograzer" (AMA) carboys received unaltered lake water, while the "high macrograzer" (HMA) treatment was created by adding enough of the zooplankton concentrate to lake water to increase initial zooplankton levels by about 4 fold (300-500 mL). Water not used in these two treatments was passed through either a 202 μm plankton net (July) or a 220 μm sieve (September and May) to remove macrozooplankton. The "low macrograzer" (LMA) carboys (which also served as the "ambient micrograzer" (AMI) treatment) were filled with water passed through this coarse sieve.

To create the remaining treatments, some of the 202 μm (or 220 μm) "filtrate" was passed through a second, 20 μm sieve. The subsequent filtrate, which had been largely cleared of micrograzers, was poured into carboys to become the "low micrograzer" (LMI) treatment, while

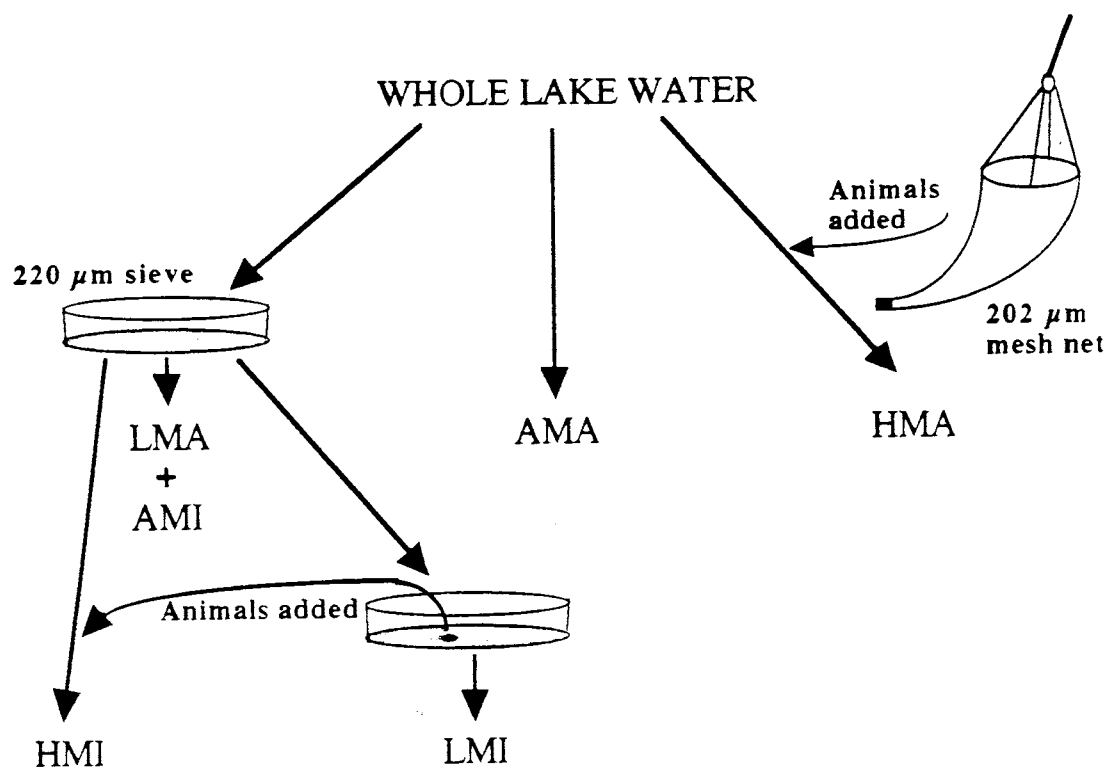


Figure 3. The procedure used to create a gradient in grazer densities in experimental carboys .

animals collected on the sieve were washed back into 20 L of 202/220 μm filtrate to produce the "high micrograzer" (HMI) treatment. To assess the potential impact of sieving on dissolved nutrient concentrations, we collected sieved and unsieved water samples for analysis of dissolved organic carbon and nitrogen (DOC and DON), and total dissolved phosphorus (TDP).

Once all the carboys were filled, KH_2PO_4 , NH_4Cl and dextrose ($\text{C}_6\text{H}_{12}\text{O}_6$) were added to each to increase P, N, and C concentrations by 3, 5 and 34 μM , respectively, thus saturating phytoplankton growth and making the nutrients supplied through grazer excretion superfluous. The carboys were incubated in Burlington Harbor suspended from anchored floating frames at a depth of 1.5 m. Incubation times were 44, 48, and 64 hrs in summer, fall, and spring, respectively. At the incubation depth, light intensities were generally below the threshold for photoinhibition for most phytoplankton groups ($<1000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$), but still high enough ($>300 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) to saturate photosynthesis.

Phytoplankton samples (60 mL samples, in triplicate) were collected from the carboys immediately before incubation, on 3-4 occasions during the first 24 h of incubation, and daily thereafter. One percent acid Lugol's solution was used as a phytoplankton preservative. Zooplankton were collected at the beginning of each experiment by sieving 10 L of the water from the mixing tank used to fill carboys through a sieve with a 64 μm (July and Sept.) or 20 μm (May) mesh, and washing the retained animals into sample bottles. These samples were used to estimate lake standing stocks. To estimate zoobiomass in individual carboys, the entire contents of each carboy was sieved (same mesh sizes as the initials) at the end of each experiment. The animals collected on sieves were anesthetized in carbonated water prior to preservation in 5% formalin with rose bengal stain.

Algal species composition and cell densities were determined by direct microscopic counts of settled samples using an Olympus inverted light microscope. The biovolume of each phytoplankton species was estimated from its cell dimensions and geometry. For common species, cell dimensions were measured during every incubation and in all treatments; scarce

species were measured during at least one incubation. Algal biomass was estimated from biovolume, using a carbon:volume conversion factor of $0.1 \mu\text{g C}\cdot\mu\text{m}^{-3}$ (Wetzel and Likens 1991).

Zooplankton were enumerated and measured under the same scope. The entire sample was counted for macrozooplankton, while subsamples were used to estimate microzooplankton numbers. Dead and moribund animals and non-grazing stages (eggs, embryos and eggs) were counted but not included in biomass estimates. The biomass of copepods and cladocerans was estimated from biomass-length relationships and that of rotifers from cell dimensions and geometry (McCauley 1984).

Phytoplankton growth rates in each carboy were determined from the regression of $\ln(\text{biomass})$ against time, and zooplankton clearance rates from the regression of growth rates on grazer biomass (done across treatments, with the macro and micrograzer treatments kept separate; see Fig. 1). The accuracy of the latter calculation is of course dependent on proper identification of herbivores. Some of our samples contained substantial densities of cyclopoid copepods, which are considered principally carnivorous, but which have occasionally been observed feeding on large algae (Reynolds 1984; Knisely and Geller 1986). We chose to compute macrozooplankton grazing rates twice, once assuming that all zooplankton graze on phytoplankton and once excluding cyclopoids. Clearance rates were determined for individual species (when abundant), phytoplankton divisions, and the phytoplankton community as a whole. To determine daily phytoplankton losses (percent loss per day) due to grazing, clearance rates were multiplied by grazer biomass (total zooplankton, or zooplankton minus cyclopoids) in the lake at the time of sampling.

Primary Productivity

Primary productivity at the water collection site was measured on the second day of the last two grazer studies (in September and May) using the ^{14}C technique and a laboratory incubator with five light intensities (as in Fee et al. 1992). A LiCor light meter and submersible probe were used to obtain information on light extinction at the sampling site, and a second meter with an

aerial probe and a data logger was used to monitor solar irradiance over the course of the grazer studies. The numerical model of Fee (1990) was employed to estimate daily primary production from the data on solar irradiance, light extinction, and productivity-light relationships. Water for the analysis was obtained in the same manner as for the grazing experiments, but fewer depths were sampled. Incubations were initiated within two hours of sample collection and were carried out at ambient temperatures. Dissolved inorganic carbon was estimated from sample alkalinity and pH. Because primary productivity is highly sensitive to day-to-day variations in cloud cover, we estimated not only the actual productivity for the days of the experiment, but also the productivity that would have occurred had the experimental days been cloudless. This allowed for more consistency between the two studies in our assessment of grazing as an avenue of C flow. I_k , one of the parameters of the photosynthesis-light relationship, may be compared with I_{ave} , the average daily light intensity within a lake's mixed layer, to assess the likelihood of light limited phytoplankton growth ($I_{ave}/I_k < 1$ suggests light limitation). We calculated I_{ave} using the equation:

$$(1) I_{ave} = (I_0/Ez) (1 - e^{-Ez}),$$

where I_0 is surface irradiance averaged over 24 hr, E is the light extinction coefficient and z is the depth of the mixing zone (Fee et al. 1992).

The Grazer vs. Nutrients Experiment

To evaluate the relative importance of grazers versus nutrients in determining Lake Champlain's phytoplankton standing stocks, we performed a 3 X 2 factorial experiment in summer 1995 (July 31- Aug. 4). Water and zooplankton were collected as in the grazing experiments (depth interval, 0-15 m) and the same "high", "ambient", and "low" macrograzer treatments created, except that each treatment was repeated in six rather than two carboys. Half of the carboys at each grazer level then were enriched with C, N and P (at the same concentrations used in the grazing experiments), while the other half were left unfertilized. Thus a total of 6 treatments were created in triplicate (HMA+, AMA+, LMA+, and HMA-, AMA-,

LMA-; += fertilizer added, - = natural nutrient levels). Chlorophyll a was the principal response variable measured. Samples for chlorophyll a were collected first from the tanks containing the zooplankton mixes used to fill carboys, and then from the carboys themselves, 23 and 92 hr into the incubation period. Phytoplankton samples also were taken from carboys at the beginning and end of the 4 day experiment, and zooplankton at the end, using the procedures described above.

Chlorophyll samples (750 mL) were filtered onto GFF glass fiber filters (effective pore size 0.7 μm) and the pigments extracted in hot ethanol (Sartory and Grobbelar 1984). The filters were allowed to leach an additional 24 h in the dark, after which the chlorophyll extract was centrifuged to remove suspended particles, and chlorophyll a absorbance was read on a spectrophotometer, using the monochromatic procedure with phaeophytin correction (Lorenzen 1967). Two-way analysis of variance was used to examine the relative contributions of nutrients and zooplankton to the final chlorophyll concentrations attained in carboys.

RESULTS

Grazing Studies

Experimental Conditions

Given that the principal goal of our study was to quantify grazing rates on important phytoplankton taxa in Lake Champlain, it was desirable that our three grazing experiments be conducted under widely different physiochemical and biological conditions. In this way, we might begin to describe an "envelope" of likely grazing loss rates for each species. As Table 1 and Figures 4 and 5 demonstrate, this objective was met. Surface water temperatures ranged from a low of 5°C in May (1995) to 17°C in September (1994) to 22°C in July (1994), while for the same periods the depth of water mixing ranged from the lake bottom (70 m at our site) to 18 m and 10 m. Nutrient concentrations were greatest in May, during spring overturn, and lowest in July, when stratification was maximal.

Phytoplankton biomass was exceptionally high during the May 1995 experiment, while the very low biomass present during the July 1994 sampling was typical of Lake Champlain's "clear water" phase. The May:September:July phytobiomass ratio was 8:2:1. While the May

Table 1. Conditions at our sampling site in Lake Champlain during the three grazing studies and the nutrients vs. grazers experiment. The chemistry data are from the VT DEC, and are for samples taken within a few days of our experiments. All values pertain to the mixed layer, and are for daytime.

Physiochemical Parameters	Grazing Experiments				Nutrients vs. Grazers
	July 1994	Sept. 1994	May 1995		July 1995
Z_m (m)	10	18	70		12
Secchi (m)	5	6.5	-		6
Temperature ($^{\circ}\text{C}$)	22	17	5		22
TP (μM)	0.32	0.29	0.35		0.29
DP (μM)	0.13	0.13	0.19		0.23
TN (μM)	29	38	49		23
DIN (μM)	12	10	-		-
TN:TP	89	130	139		79
DIN:TP	36	33	-		-
DSi (μM)	2.9	25	-		8.2
Biological Parameters					
Chlorophyll a ($\mu\text{g L}^{-1}$)	1.5	2.1	6.3		1.8
Phytoplankton Biomass ($\mu\text{g dwt L}^{-1}$)	34	61	278		50
Zooplankton Biomass ($\mu\text{g dwt L}^{-1}$)	79	1282	695		1144
Grazer Biomass ($\mu\text{g dwt L}^{-1}$)	79	371	485		648
Prim. productivity ($\text{mg C m}^{-2} \text{d}^{-1}$)	-	349	1660		-
C Turnover in Phytoplankton (d)*		1.6	5.9		

* Assumes that phytoplankton are 50% C.

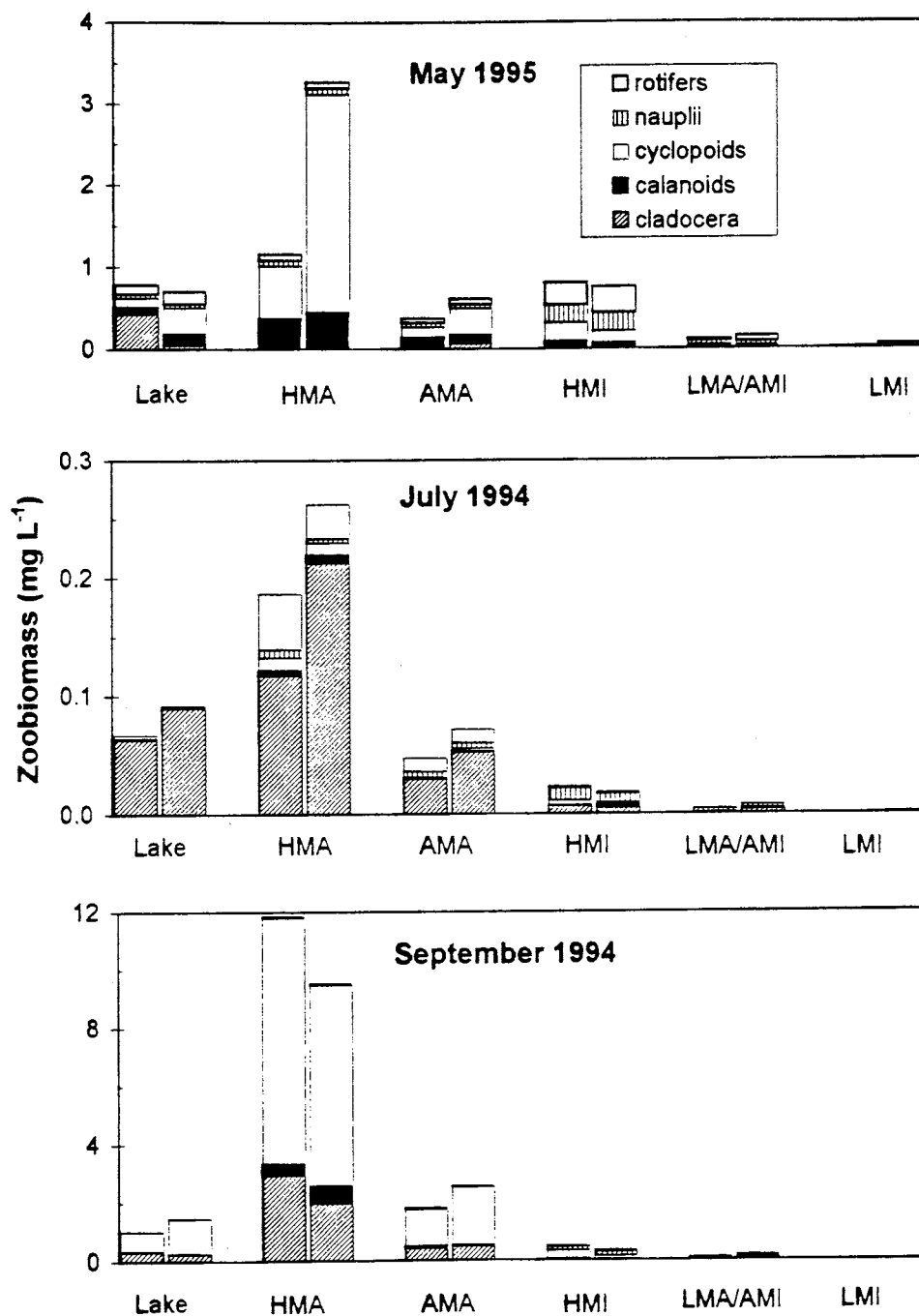


Figure 4. The biomass of major zooplankton groups in experimental carboys at the end of the three grazing studies. The "lake" samples were taken at the initiation of the experiments from the 240 L tank used to fill carboys. Lack of rotifers and nauplii in the "lake" in July is due to these two samples accidentally being concentrated with a 90 μm rather than a 64 μm sieve prior to counting; the sieve probably allowed rotifers to pass.

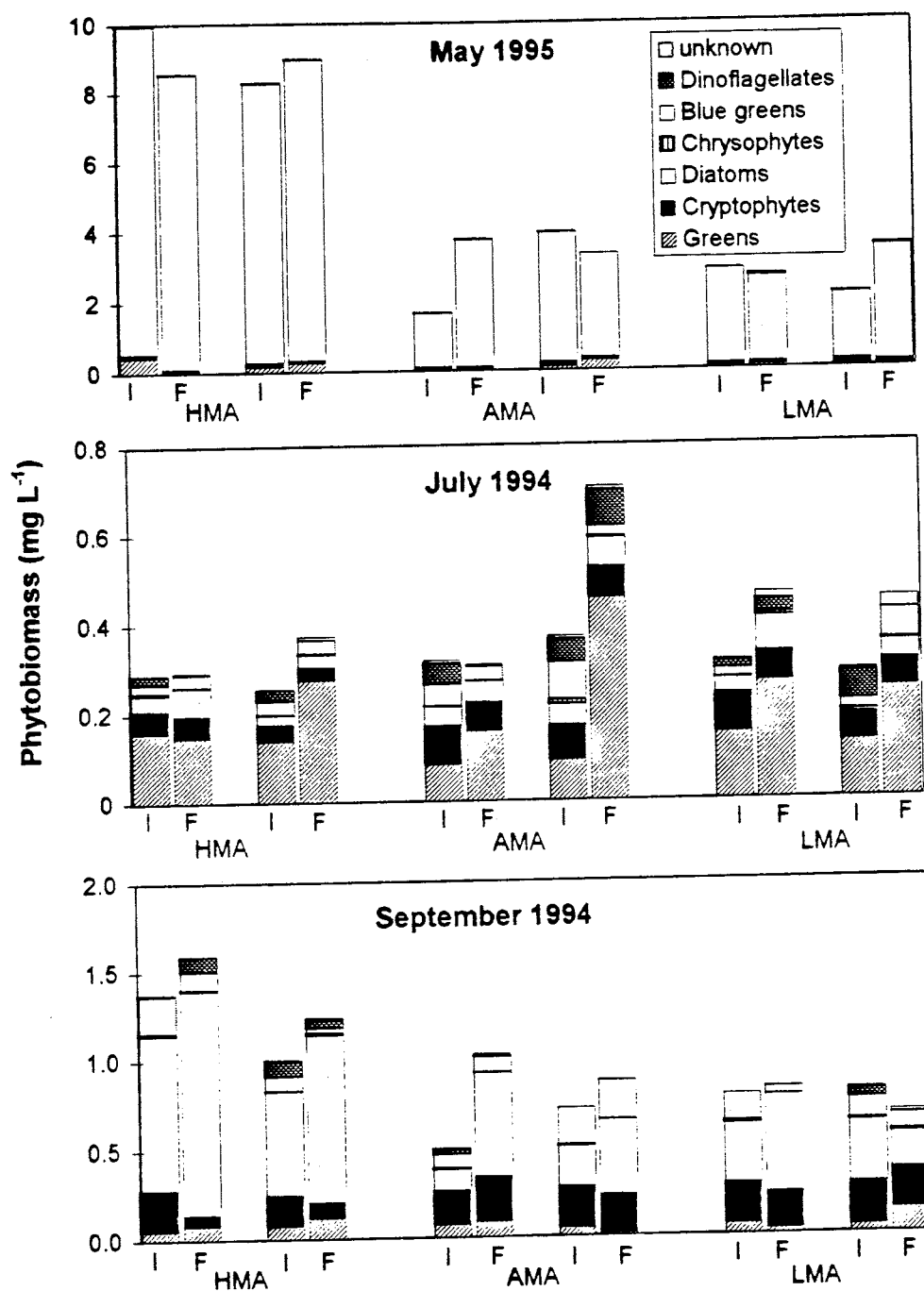


Figure 5. The biomass of different phytoplankton groups in experimental carboys at the beginning and end of the grazing experiments. Three levels of macrozooplankton density were used. Incubation times were in 44, 48 and 64 h in July, October and May, respectively. Two additional samples were taken between the times shown for use in calculating phytoplankton growth rates.

phytoplankton community was strongly dominated by diatoms (96% of biomass) and especially by the genus *Melosira* (67% of phytobiomass), the July community was composed largely of cryptophytes (especially *Chroomonas* and *Cryptomonas*) and green algae (e.g., *Mougeotia*, *Oosystis* and *Eudorina*). The September community was intermediate between the other two samplings in its composition (a mixture of cryptophytes, green algae and diatoms).

The zooplankton community was most diverse during the May diatom bloom, consisting of a mixture of cladocerans (especially *Daphnia retrocurva*, and *Daphnia galeata mendotae*), cyclopoid copepods, and calanoid copepods in nearly equal proportions. By contrast, the July community consisted almost entirely of cladocerans, while the September community was dominated by cyclopoid copepods, with *Acanthocyclops robustus* the best represented species. Zooplankton biomass was greatest in fall and minimal in summer. The ratio of September: May: July zooplankton biomass was 16:9:1. Although cladocerans were almost the only zooplankton present in the epilimnion in July, they were less abundant than during the other samplings. The ratio of herbivore biomass among the three samplings (cyclopoid copepods eliminated from the estimate) was 5:6:1.

Primary Productivity

Our study provided the first estimates of primary productivity in Lake Champlain. Because of the novelty of the data, we present in Table 2 the photosynthetic parameters obtained. α^B is the rate at which photosynthesis (per mg of chlorophyll a) increases with light intensity (PAR) at low light intensities (the slope of the linear portion of a plot of photosynthesis per mg chlorophyll a vs. light intensity), while P_m^B is the rate of photosynthesis per mg of chlorophyll a at light saturation (the plateau of the curve), I_k (P_m^B/α^B) is the light intensity below which photosynthesis is primarily light limited, and P_{opt} is the product of P_m^B and chlorophyll a concentration. Both P_m^B and I_k were lower during the May than during the September grazing study, indicating that the spring phytoplankton were more narrowly adapted to low light intensities than the community present in fall. Daily insolation was greater in May than in

Table 2. Light and photosynthesis parameters for Lake Champlain during the grazing experiments conducted during September 1994 and May 1995.

Parameter	Sept. 1994	May 1995
Light extinction coefficient (m^{-1})	0.76	0.41
Mixed layer depth (m)	18	70
I_{ave} (cloudless) ($\text{Ei m}^{-2} \text{h}^{-1}$)	0.13	0.11
P_m^B ($\text{mg C mg chl a}^{-1} \text{h}^{-1}$)	7.9	2.7
a^B ($\text{mg C mg chl a}^{-1} \text{Ei m}^{-1}$)	10.2	5.5
I_k ($\text{Ei m}^{-2} \text{h}^{-1}$)	0.49	0.77
$I_{\text{ave}}: I_k$	0.3	0.1
Specific Prim. Prod. ($\text{mg C mg chl a}^{-1} \text{d}^{-1}$)	10.2	3.2
Chlorophyll a (mg m^{-3})	1.9	7.4
Prim. Prod. ($\text{mg C m}^{-2} \text{d}^{-1}$)	349	1660
Prim. Prod. ($\text{mg C m}^{-3} \text{d}^{-1}$)	19	24

September and the rate of light extinction lower. Thus I_{ave} , the average daily light intensity over the mixing depth was similar for both studies, despite the much greater depth of mixing in May (70 vs. 18 m). For both studies I_{ave}/I_k was substantially <1 , suggesting light limitation during these mixing periods. Specific primary productivity (productivity per mg of chlorophyll a) was substantially greater in September than May (10.2 vs 3.2 mg C·mg chl a $^{-1}$ ·d $^{-1}$), but total productivity over the water column (assuming a cloudless day) was much greater during the spring study (1660 vs 349 mg C m $^{-2}$ ·d $^{-1}$), due to the deeper lighted water column. The turnover time of C in algae (standing stock over the depth of the mixed layer divided by C fixation rate) was 5.9 d in May compared with 1.6 d in September.

Success of the Manipulations

Our method for estimating macrozooplankton grazing rates required that natural levels of microzooplankton and phytoplankton be maintained across treatments and that the integrity of the macrozooplankton community be preserved (i.e., species composition not altered by sieving and addbacks). These requirements were reasonably well met during the July and September studies (Figs. 4, 5). In May, however, colonial diatoms dominated the phytoplankton. While the individual cells were not large, many colonies were large enough to be retained by our 202 μ m net. Consequently, the HMA treatment contained 2-3 times the phytoplankton biomass present in the AMA and LMA treatments. Given that zooplankton clearance rates are generally inversely proportional to food density except at saturating food concentrations, this methodological error may have resulted in an underestimation of grazing rates for the May experiments (i.e., in slope lowering).

Another problem with the May experiment was related to cyclopoid copepod abundance. These animals were three times more abundant in one of the two HMA carboys than in the other. Furthermore, the AMA duplicates differed with regard to whether calanoid or cyclopoid copepods were more important. Because cyclopoid copepods are believed to be largely carnivorous, differences in their abundance may not directly violate our requirement of similar grazer

composition among carboys. However, cyclopoids might affect grazing indirectly by feeding on herbivores. Poor replication of grazer biomass within treatments (Fig. 4) was not an issue, as data from each carboy were entered separately into regression analysis.

Meeting experimental prerequisites was a greater problem for the micrograzer than the macrograzer portion of the study. Although our 20 μm sieve removed >95% of macrograzer biomass from the water used in the micrograzer treatments, a few larger animals managed to slip through the mesh (or somehow went around it). Microzooplankton were minor components of the zooplankton community during these experiments. Consequently, contamination by a macrozooplankton seriously affected the total biomass of grazers present (Fig. 4). Macrograzers made up from 13-57% of the biomass in the AMI treatments, and 36-44% of that in the HMI treatments (the sieve used to concentrate micrograzers for add-backs also concentrated the unwanted macrograzers). Macrograzer contamination at a constant level of total biomass would have allowed the calculation of micrograzer clearance rates to proceed without reservation. However, this was not the case: macrograzer biomass was 3-7X greater in the HMI than AMI carboys, and macrograzer biomass in the AMI carboys was an order of magnitude greater than in the LMI carboys (a lone macrograzer was found in a couple carboys).

The micrograzer experiments were further compromised by serious alteration of phytoplankton communities during sievings to remove zooplankton nettings that added animals (Fig. 6). The HMI carboys contained about three times as much and the LMI carboys only one half the phytobiomass in the lake (AMI). Cryptophytes were small enough to pass through the 20 μm sieve and thus avoid manipulation, but green algae, diatoms and most blue green species were concentrated by the 20 μm sieve.

Phytoplankton Response to Manipulations and Grazing Rates

Because all of the experimental carboys received nitrogen and phosphorus amendments and these two nutrients generally limit algal biomass in the lake (Levine et al. 1997), we expected two different responses to grazer manipulations: 1) an increase in the biomass of phytoplankton

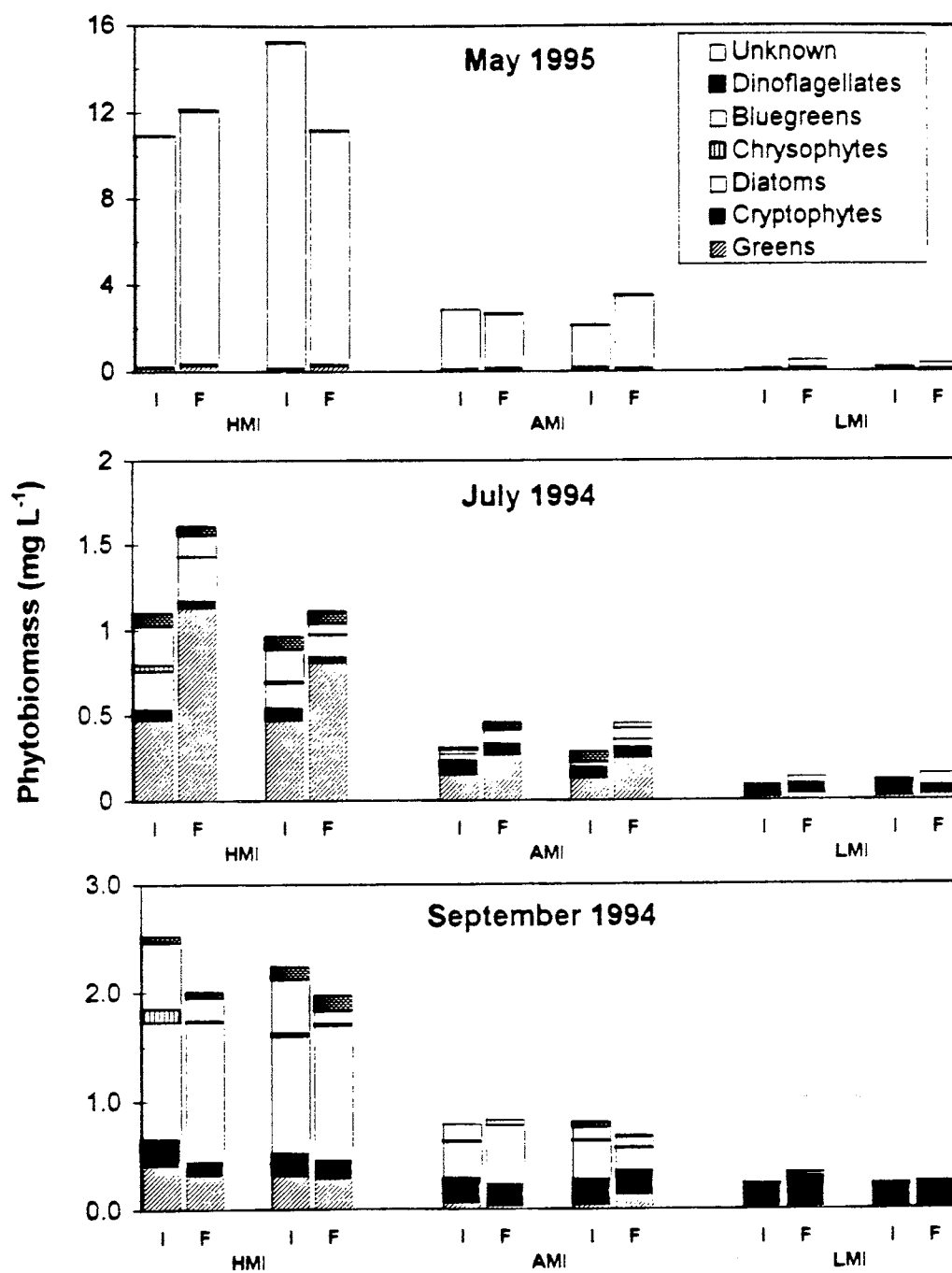


Figure 6. The biomass of different phytoplankton groups in experimental carboys at the beginning and end of the grazing experiments. Three levels of microzooplankton density were used. Incubation times were 44, 48 and 64 h in July, October and May, respectively.

relatively resistant to grazing as these species exploited the nutrients, and 2) biomass declines among edible phytoplankton exposed to high densities of grazers. In fact, the short duration of the three grazing experiments (2-3 days) resulted in only small net changes in total phytoplankton biomass and species composition (Figs 5, 6). During the July study, green algae increased in the carboys relieved of macrozooplankton grazing pressure (LMA and HMI; not LMI, as green algae were largely removed by the 20 μ sieve), bringing up overall phytoplankton biomasses in these systems as well. During the September study, diatoms increased in the AMA and HMA carboys, while, in some of them, blue-green algae and cryptophytes declined (the green algal response was inconsistent). In May, no response to nutrient addition occurred; phytoplankton biomass remained level, and diatoms dominant. Nutrient enrichment experiments carried out a week after this grazing study showed a lack of growth limitation by any of the nutrients normally scarce in the lake (N, P, and Si; Levine et al. 1997).

The initial and final phytoplankton densities shown in Figs. 5 and 6 were combined with the densities obtained at intermediate time points to arrive at the estimates of grazing rates shown in Tables 3-6. As expected, the clearance rate estimates that included cyclopoid copepods as grazers were often much lower than those excluding these animals (the large increase in grazer biomass diluted specific grazing rates). Very few cyclopoids were present to affect the estimates for July 1994, but for the September 1994 and May 1995 experiments, clearance rates excluding cyclopoids were 3 and 5 times greater than those including these animals. Percentage loss rates were more comparable because the lower clearance rates obtained including cyclopoids were multiplied by greater animal biomasses to arrive at the loss estimates.

Discrepancies in taxon vulnerability to macrograzers were apparent, both at the group level (Table 4) and among species within a group (Table 5). Some estimates of clearance rates were negative, indicating that the phytoplankton species involved grew more rapidly in the presence than in the absence of grazers, probably because they were relatively immune to predation and benefited from reduced competition. During the July study, dinoflagellates lost 15% of their standing stock to grazing per day, while green algae lost 6%, and cryptophytes 1%.

Table 3. Clearance and grazing rates for macrozooplankton feeding on phytoplankton of all sizes, and for microzooplankton feeding on phytoplankton <20 μm in size. For the macrozooplankton, rates are given both for confirmed herbivores (cyclopoids excluded) and for the total zooplankton community.

		Clearance ($\text{mL} \cdot \text{day}^{-1} \cdot \text{g dwt zoopl.}^{-1}$)					
		July 1994		Sept. 1994		May 1995	
		Herbivores	Total Zoo.	Herbivores	Total Zoo.	Herbivores	Total Zoo.
Macrozooplankton		0.06	0.06	-0.02	0.00	0.26	0.04
Microzooplankton		1.47		0.49		0.76	
							0.00
						0.17	-

		Percent removal per day					
		July 1994		Sept. 1994		May 1995	
		Herbivores	Total Zoo.	Herbivores	Total Zoo.	Herbivores	Total Zoo.
Macrozooplankton		0.4	0.4	0.0	0.0	10	3
Microzooplankton		1.4		1.7		11	
Total		2.4	2.4	1.7	1.7	21	14
						11	0
						-	-

* Clearance rates estimated from chlorophyll a concentrations were similar to these estimates from phytoplankton, 0.18 and 0.00 $\text{mL} \cdot \text{day}^{-1} \cdot \text{g dwt}^{-1}$.

** For chlorophyll a, the removal rates were also 11 and 0% per day.

Table 4. Macrozooplankton grazing rates on specific phytoplankton taxa, expressed as clearance rates and as percentage of prey removed per day. Rates are given both for confirmed herbivores and for the entire zooplankton community (including cyclopoids). Rates were not calculated for algal divisions containing <10% of phytoplankton biomass. * indicates that a clearance rate was significantly different from zero at $p < 0.05$.

Clearance ($\text{mL day}^{-1} \text{ g dwt zoopl.}^{-1}$)									
Division	July 1994		Sept. 1994		May 1995		July 1995		
	Herbivores	Total Zoo.	Herbivores	Total Zoo.	Herbivores	Total Zoo.	Herbivores	Total Zoo.	
Greens	0.92	0.92	-	-	-	-	0.41*	-0.05	
Cryptophytes	0.12	0.12	0.18*	0.05*	-	-	0.13*	0.00	
Diatoms	-0.68	-0.68	-0.01	0.00	0.19	0.031	0.08	0.00	
Blue greens	-0.39	-0.39	0.15	0.04	-	-	0.07	-0.12	
Dinoflagellates	2.11	2.11	-	-	-	-	-	-	

Percent removal per day									
Division	July 1994		Sept. 1994		May 1995		July 1995		
	Herbivores	Total Zoo.	Herbivores	Total Zoo.	Herbivores	Total Zoo.	Herbivores	Total Zoo.	
Greens	6	6	-	-	-	-	26	0	
Cryptophytes	1	1	7	6	-	-	8	0	
Diatoms	-	-	0	0	10	2	5	0	
Blue greens	0	0	6	5	-	-	4	0	
Dinoflagellates	15	15	-	-	-	-	-	-	

Table 5. Clearance and percent removal rates for macrozooplankton grazing on specific species of phytoplankton. Rates are calculated for both removal by confirmed herbivores and by all zooplankton (including cyclopoids). Although genus names are given, the calculations are generally for individual species.
 * indicates that a clearance rate was significantly different from zero at $p < 0.05$.

Clearance (mL day ⁻¹ g dwt zoopl. ⁻¹)								
Species	July 1994		Sept. 1994		May 1995		July 1995	
	Herbivores	Total Zoo.	Herbivores	Total Zoo.	Herbivores	Total Zoo.	Herbivores	Total Zoo.
Mougeotia	1.8	1.8	-	-	-	-	-	-
Coelastrum	-	-	1.8*	0.51*	-	-	-	-
Cryptomonas	1.79*	1.79*	0.28	0.08*	-	-	0.46	0.01
Chroomonas	-0.59	-0.59	0.06	0.02	-	-	0.09	0.00
Aphanizomenon	-	-	-0.05	-0.05	-	-	-	-
Chroococcales	-	-	-	-	-	-	0.14	-0.01
Fragilaria	-	-	0.00	0.00	-	-	-	-
Tabellaria	-	-	-0.01	-0.01	-	-	-	-
Melosira	-	-	-	-	0.16	0.03	-	-
Centrales [†]	-5.63	-5.63	-	-	-	-	0.05	0.04

Percent removal per day								
Species	July 1994		Sept. 1994		May 1995		July 1995	
	Herbivores	Total Zoo.	Herbivores	Total Zoo.	Herbivores	Total Zoo.	Herbivores	Total Zoo.
Mougeotia	13	13	-	-	-	-	-	-
Coelastrum	-	-	58	64	-	-	-	-
Cryptomonas	12	12	9	10	-	-	28	1
Chroomonas	0	0	2	2	-	-	6	0
Aphanizomenon	-	-	0	0	-	-	-	-
Chroococcales	-	-	-	-	-	-	9	0
Fragilaria	-	-	0	0	-	-	-	-
Tabellaria	-	-	0	0	-	-	-	-
Melosira	-	-	-	-	8	2	-	-
Centrales [†]	0	0	-	-	-	-	3	4

† An unidentified, small species, not colonial. This group does not include Melosira.

(These estimates, and all estimates referred to heretofore are based on exclusion of cyclopoids from the grazer biomass estimate.) Bluegreen algae had negative clearance rates, and thus probably were largely invulnerable to grazing. In September, when dinoflagellates and green algae were scarce, cryptophytes and blue green algae were subject to greater grazing mortality, 7 and 6% day⁻¹, respectively. Diatoms also were present at this time, but apparently not utilized by grazers (loss rate, 0% per day). In May, diatoms, which totally dominated the phytoplankton, lost an average of 7% of their biomass per day to grazers.

Table 5 shows grazing rates for a small selection of phytoplankton species (genus names are listed, but most represent a single species). While data were available to calculate grazing for many more species, only the species shown were present at densities great enough that we felt it might be possible to distinguish grazer impacts from natural spatial and sampling variability. We found that, among the cyrtomonads, *Cryptomonas* was more vulnerable to predation (11-12% loss per day) than *Chroomonas*, the most abundant species in the lake on an annual basis (loss rate, 0-2% per day). The two differed species differed principally in size; *Cryptomonas* was almost twice as long as *Chroomonas* (7 vs 4 μm , on average) and had twice the latter's volume (25 vs. 12 μm^3). the Among bluegreen algae, Chroococcales were more heavily grazed than *Aphanizomenon*, and among diatoms, *Melosira* suffered greater grazing losses than *Fragilaria* and *Tabellaria*. For the phytoplankton community as a whole (total standing stock), macrograzer clearance rates were low. Our estimates of overall grazing loss rates during the July and September experiments were <1% per day, while the loss rate in May was about 10% per day.

Few of the clearance rates that we obtained were statistically significant from zero at a p level of 0.05. This situation was partly related to the fact that loss rates truly were low over the period examined and also to the patchy distribution of phytoplankton and zooplankton in water samples. Colonial forms like *Melosira* and *Mougeotia* result in variable cell counts between replicate samples and thus lower the r^2 values for regression curves. Inclusion or omission of a single exceptionally large zooplankter also skews biomass estimates. Because of the low t statistics obtained, the grazing rates reported here must not be viewed as absolutes, but as rough

Table 6. Microzooplankton grazing rates for specific phytoplankton groups expressed as clearance rates and as percentage of prey removed per day. Rates were not calculated for algal divisions containing <10% of phytoplankton biomass. * indicates that the rate is significantly different from zero at $p < 0.05$.

Division	Clearance (mL day ⁻¹ g dwt zoopl. ⁻¹)			Percent Removed per Day		
	July 1994	Sept. 1994	May 1995	July 1994	Sept. 1994	May 1995
Greens	-0.22	-	-	0	-	-
Cryptophytes	2.48	1.87	-	2	6	-
Diatoms	-	0.49	1.16	-	2	17
Blue greens	-	2.19	-	-	7	-
Dinoflagellates	-	-	-	-	-	-

estimates of grazing trends.

Greater clearance rates were measured for micrograzers feeding on cryptophytes, diatoms and blue green algae than for macrograzers consuming the same groups (Table 6 vs. Table 4). Green algae appeared to be grazed less efficiently by micrograzers than by macrograzers, however. Our estimate of total grazing mortality on phytoplankton due to micrograzers was similar to that incurred by macrograzers. While macrograzers accounted for considerable more biomass than micrograzers in the lake, the higher clearance rates of the latter group appeared to compensate for the lower biomass.

If the grazing rate estimates that we obtained are accepted at face value (despite the low values and all our reservations about the validity of the micrograzer study), it can be concluded that 2-21% of phytoplankton biomass was lost each day to zooplankton grazing. If it is assumed that phytoplankton are 50% carbon, then it can also be concluded that this grazing loss balanced 3% of primary productivity in September, but the entire lot (117%) in May.

Grazers vs. Nutrients

The grazing vs. nutrients experiment took place at the same time of year as the July grazing experiment, but a year later. Epilimnion depth, temperature, nutrient concentrations and phytobiomass were nearly identical during the two studies, while the phytoplankton communities differed only in that blue green algal abundance was slightly greater in 1995 (Table 1). There was one major difference in the conditions of the two studies however: zooplankton biomass was 15X greater during 1995 than during 1994 (Fig. 4 vs. 7). Furthermore, while the 1994 zooplankton community was cladoceran dominated, the 1995 community contained a mixture of cladocerans and copepods.

Chlorophyll a concentration was the principal phytoplankton response variable measured during this experiment; phytoplankton enumeration was restricted to the fertilized carboys and to initial and final samplings. Samples taken 24 hours into the experiment, indicated little change in chlorophyll a concentrations relative to initial values (Fig. 8), and suggested that phytoplankton

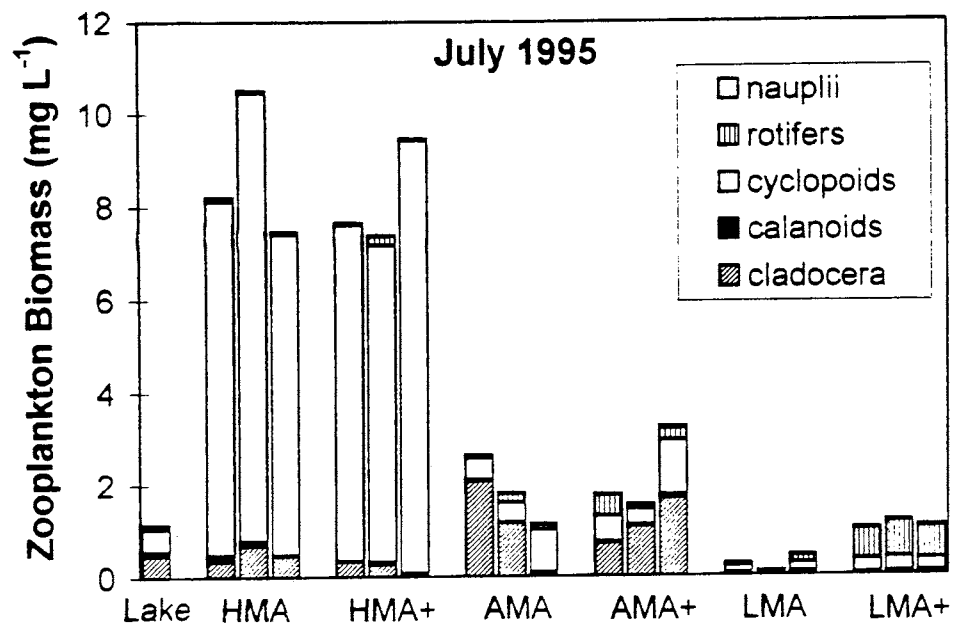


Figure 7. The biomass of major zooplankton groups in experimental carboys at the end of the nutrients vs. grazers experiment.

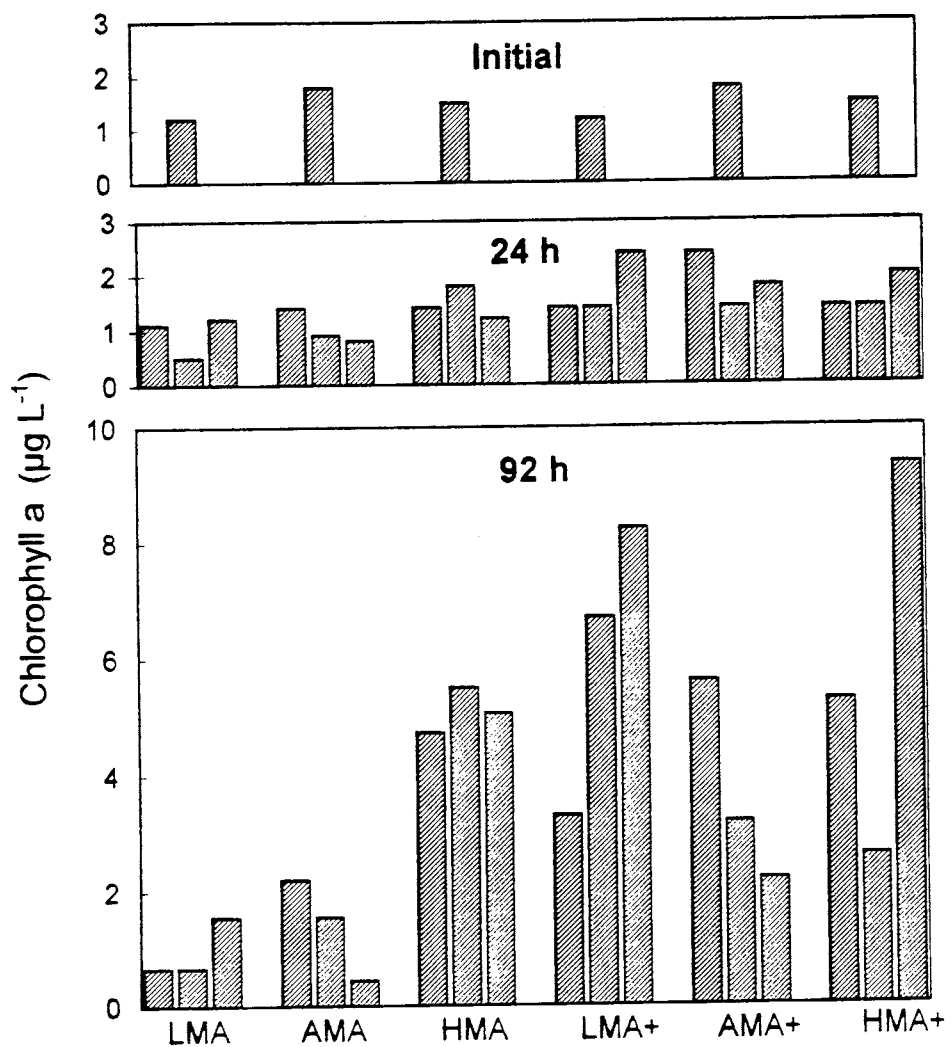


Figure 8. Chlorophyll a concentrations in experimental carboys at 0, 24 and 92 h during the nutrients vs. grazers experiments. Initial concentrations were measured for the water used to fill carboys of similar treatment.

may need a day or more to "gear up" for nutrient assimilation and division (Reynolds 1984), and/or recovery from handling. After 92 hours, however, a considerable divergence in chlorophyll a concentrations had occurred. Most carboys that received nutrients showed an increase in pigment (and phytoplankton biomass, Fig. 8), while those that contained zooplankton at or below ambient densities and did not receive nutrients showed no increase in chlorophyll a. The "surprise" of the experiment was elevated chlorophyll a concentrations in the unfertilized carboys amended with zooplankton at several times ambient levels. Two-way ANOVA indicated that the impact of nutrient addition on chlorophyll levels was highly significant ($p=0.01$), while the impact of zooplankton density was less significant ($p=0.06$) and no nutrient X zooplankton interaction was apparent.

Analysis of zooplankton communities at the end of the experiment, however, indicated that the grazer treatments were not entirely what we expected them to be. Total zooplankton and cyclopoid biomass increased along the gradient LMA to AMA to HMA, as was expected, but cladoceran biomass peaked in the AMA carboys and rotifer biomass in the LMA carboys (Fig. 7). Since cladocerans and rotifers are mostly herbivores, while cyclopoids are mostly carnivores, this trend made the results of the experiment difficult to interpret. To what extent the discrepancies were due directly to our manipulations versus due to carnivore-herbivore interactions is unknown.

A plot of increase in chlorophyll a concentration during the experiment against total zooplankton biomass (grazers and cyclopoids) revealed no relationship between the two variables in the presence of added nutrient, but a strong positive relationship in its absence (Fig. 9). When the same variable was plotted against grazer biomass (cyclopoids not included) a different relationship was apparent: chlorophyll a concentration declined sharply with grazer biomass in the fertilized systems, but in the unfertilized systems first increased and then decreased with grazer biomass. These trends probably result from the conflicting impacts of grazing mortality and zooplankton-mediated nutrient regeneration on phytoplankton standing stocks.

We took advantage of the gradients in macrograzer densities created in fertilized carboys to obtain an additional set of grazing rate estimates for mid-summer (Tables 3-5). Because the

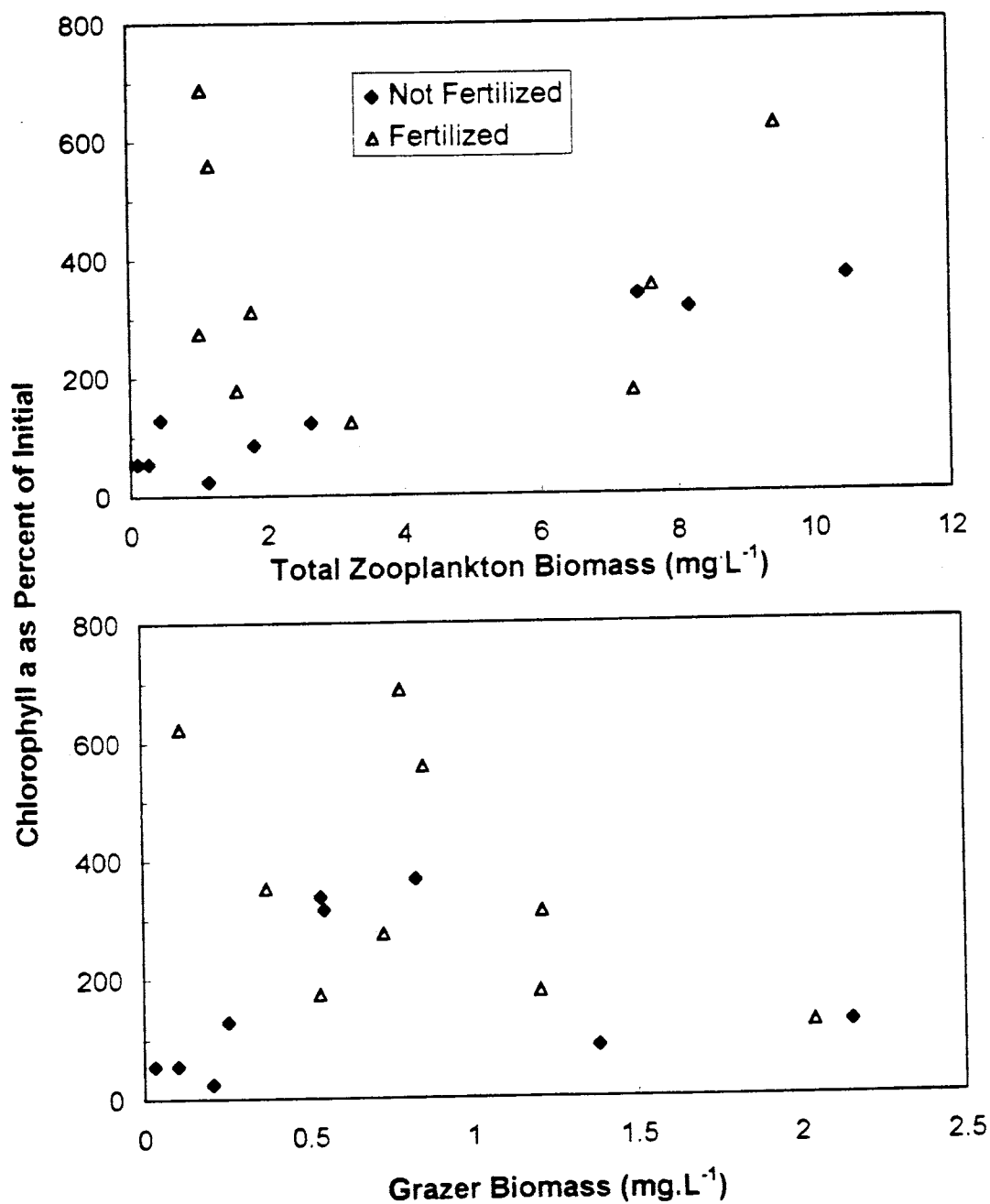


Figure 9. Chlorophyll a concentration as a percentage of the initial value versus (a) zooplankton biomass and (b) grazer biomass (cyclopoids excluded).

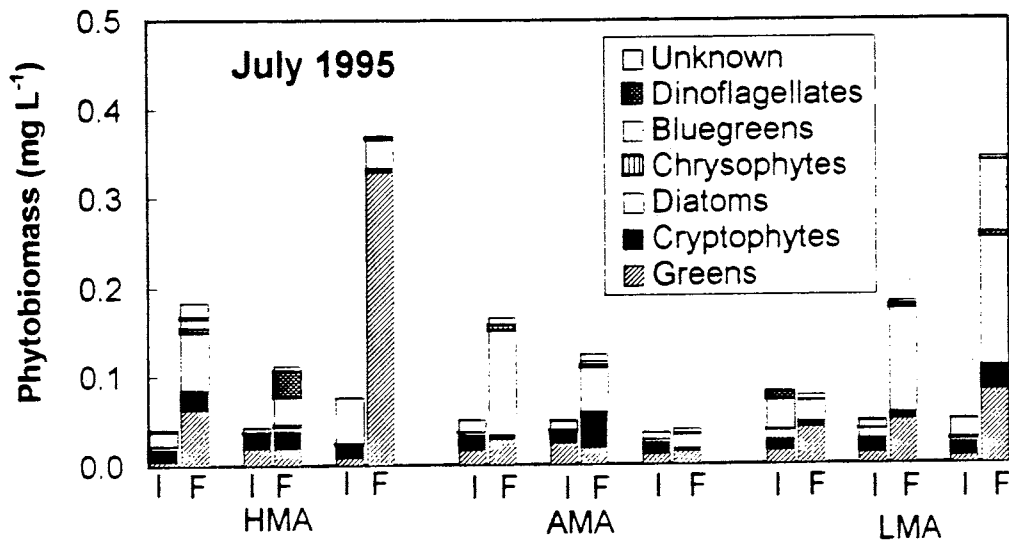


Figure 10. The biomass of major phytoplankton groups in experimental carboys at the end of the nutrients vs. grazers experiment.

nutrients vs. grazers study was longer (4 day) than the grazing experiments (2-3 days), phytoplankton response to nutrient addition was much more apparent (Fig 10), and more of the calculated clearance rates were significantly different from zero. Otherwise, our findings were similar to those obtained during the previous year: green algae appeared again to be more heavily grazed than diatoms, cryptophytes and blue green algae (26% vs 4-8% of biomass per day), and, again the cryptophyte *Chroomonas* escaped grazing mortality relative to the larger *Cryptomonas* (6% vs 28% loss per day). The net clearance rate on all phytoplankton was estimated to be 0.17 ml per g of zooplankton per day, or 11% of phytoplankton per day. An estimate of net clearance rate based on change in chlorophyll a concentrations in the fertilized carboys over the 4 day incubations yielded nearly identical results ($0.18 \text{ ml} \cdot \text{g zooplankton}^{-1} \cdot \text{day}^{-1}$ and 11% of biomass per day). All of these estimates assume that cyclopoids did not graze. If these very abundant animals are included, all grazing rate estimates for the experiment are near zero.

DISCUSSION

Macrozooplankton Grazing in Lake Champlain

Analysis of grazing on phytoplankton in Lake Champlain revealed substantial variability among the phytoplankton with regard to their vulnerability to macrozooplankton. Some species were grazed at rates that depleted 10-25% of their biomass every day, while others were grazed at more moderate rates, or not at all. Some species grew more rapidly in carboys amended with zooplankton than in carboys containing fewer zooplankton, and thus had "negative grazing rates". These algae probably were immune to grazing and had better access to resources when their more vulnerable competitors were suppressed. There are many reports of similar algal stimulation by grazer addition in the literature (e.g., Lehman and Sandgren; Berquist and Carpenter 1986; Elser and Goldman 1991; Elser 1992).

Grazing rate estimates made at the division level suggested that the phytoplankton groups in Lake Champlain most vulnerable to grazing are the dinoflagellates and green algae. Cryptophytes appeared to be intermediate in vulnerability, while diatoms and blue-green algae

were only sparingly grazed. Analysis of species-specific rates indicated, however, that division-level grazing rates mask important differences in grazing mortality among the species within the divisions. For example, in the three experiments in which *Cryptomonas* and *Chroomonas* were present, the former consistently was removed from the water at a rate roughly four times the rate of removal of the latter, and at a clearance rate as high as the rates measured for green alga and dinoflagellate removal. It was only because the poorly grazed *Chroomonas* is generally much more abundant than *Cryptomonas* in Lake Champlain that we found cryptophytes as a group to be less than vigorously grazed. Among the blue green algae, we estimated greater grazing on Chroococcales than on *Aphanizomenon*, and, among the diatoms, more grazing on *Melosira* than on *Fragilaria*, *Tabellaria* and a small-celled species of Centrales.

Many of our conclusions about taxon-based differences in vulnerability to grazing were in agreement with previous findings and with widely-accepted concepts about the role that grazers play in influencing algal species composition; a few were not. One important premise of phytoplankton ecology is low grazing pressure on blue green algae, which may result from the large size of these algae when present as filaments or colonies (Lampert 1981; Infante and Abella 1985), or from toxicity (Arnold 1971), unpalatability (Porter and Orcutt 1980) or low nutrient content (Lampert 1981). Blue green algal blooms in eutrophic lakes have been partially explained by the ability of blue green algae to escape predator control and thus monopolize nutrient supplies (e.g., Hrbacek 1964; Porter 1977; Sommer et al. 1986). Our estimates of blue green algal removal through grazing were minimal (<1% per day) across the three experiments in which the group was present. However, a number of researchers have shown that zooplankton will eat blue green algae when more desirable algae are unavailable (e.g., de Bernardi 1981, Schoenberg and Carlson 1984).

Dinoflagellates also are presumed to be relatively immune to grazing because of their frequent large size (Porter 1977; Sommer et al. 1986). The species present during our studies were relatively small (except for *Ceratium hirundinella*) and were more strongly grazed than any other group. This finding is not unique. The *Gymnodinium* present in Elser's (1992) study were

grazed at a rate similar to that which we record here. Also, Proulx et al. (1996) found that dinoflagellates and cryptophytes were the main groups to respond positively to zooplankton removal through heavy fish predation in a Quebec lake.

Among the phytoplankton generally considered most vulnerable to grazers are cryptophytes, chrysophytes, and small non-colonial diatoms (Porter 1977; Sommer et al. 1986). Chrysophytes were too rare in Lake Champlain during our studies to permit estimation of their losses to grazers, while the response of cryptophytes to grazing was mixed. *Chroomonas* was meagerly grazed, while *Cryptomonas* sp. was one of the most vulnerable species. Many researchers have reported on the apparently high edibility of the latter genus (e.g., Porter 1977; Geller 1984, Lehman and Sandgren 1985, Knisely and Geller 1986, Bogdan and Gilbert 1987, Cyr and Pace 1992). The *Cryptomonas* clearance rates measured by Elser and Goldman (1991) and Cyr and Pace (1992) were even greater than those reported here.

The diatoms common in Lake Champlain are mainly colonial species. Thus our finding of minimal grazing on diatoms in general, and on all species examined except *Melosira* (which was grazed at a rate of 7% per day in May 1995), concurs with current ideas about diatom grazability. Elser and Goldman (1991) also reported very low grazing rates on diatoms in the California lakes that they studied.

Green algae are a highly diverse group which apparently contains both inedible and edible species (Porter 1977; Sommer et al. 1986). We found this group to be one of the most heavily grazed in Lake Champlain, although some of the algae used as prey items (e.g. *Mougeotia*) have been previously classified as inedible (Knisely and Geller 1986). In their study of California lakes, Elser and Goldman (1991; Elser 1992) measured low grazing rates on most green algae, but relatively high rates for certain species of *Cosmarium*, *Oocystis*, *Quadrigula* and *Selenastrum*.

We observed no obvious distinguishing features between phytoplankton species that were vulnerable versus immune to grazing. Because *Chroomonas* and the unidentified Centrales species that we examined were both small ($<4\ \mu\text{m}$ across; biovolume $<13\ \mu\text{m}^3$) and poorly grazed relative to other species in their divisions, we hypothesized that small size might be a factor in

factor in their poor removal from the water. A number of laboratory studies have shown that grazing rates are dependent on food size (e.g., Gliwicz 1977, Vanderploeg 1981, Vanderploeg et al 1988). Very small particles can slip through filtering setae, while very large particles may not fit through the gap in a cladoceran carapace or through the mouth of either cladoceran or copepod (Gliwicz 1977). While many cladocerans are capable of filtering particles as small as bacteria from the water, their filtering efficiency is greater for intermediate sized particles (Svensson and Stenson 1991). Most studies to date have focused on the handling of large particles, and have concluded that phytoplankton $>35\text{ }\mu\text{m}$ across are only marginally grazed (Lampert et al. 1986; Svensson and Stenson 1991). However, very few phytoplankton of this size are found in Lake Champlain. None were abundant during our studies. To assess the impact of phytoplankton size on grazing, we made a plot of the species specific loss rates that we obtained over the four experiments against the greatest linear axial dimension (GALD) of each species. This plot suggested a tendency for grazing to decline at $\text{GALD} < 5\text{ }\mu\text{m}$, but the impact of large size ($>30\text{ }\mu\text{m}$) on reducing ingestion was more pronounced (Fig. 11). The laboratory feeding experiments of Svensson and Stenson (1991) and Knisely and Geller (1986) indicate optimal feeding by both cladocerans and rotifers between 3 and 20 μm .

The overall impact of grazing by macrograzers in the lake (0-11% day) was comparable with most loss rates reported in the literature. While there are occasional reports of phytoplankton grazing loss rates of 100-400% per day (Haney 1973; Gliwicz and Hillbricht-Ilkowska 1972; Jarvis 1986; Lampert et al. 1986), more than 75% of the values reported in the literature suggest grazing losses of $<50\%$ per day (Cyr and Pace 1992). Cyr and Pace (1992) estimated grazing losses of $<10\%$ in two-thirds and $<5\%$ in half of the 30 phytoplankton assemblages (16 lakes) which they examined in grazing studies. Some of the higher estimates of grazing losses in the literature are due to the fact that grazing was estimated by adding labelled cells to feeding chambers and measuring their incorporation into zooplankton. Such studies usually use phytoplankton with an above average edibility.

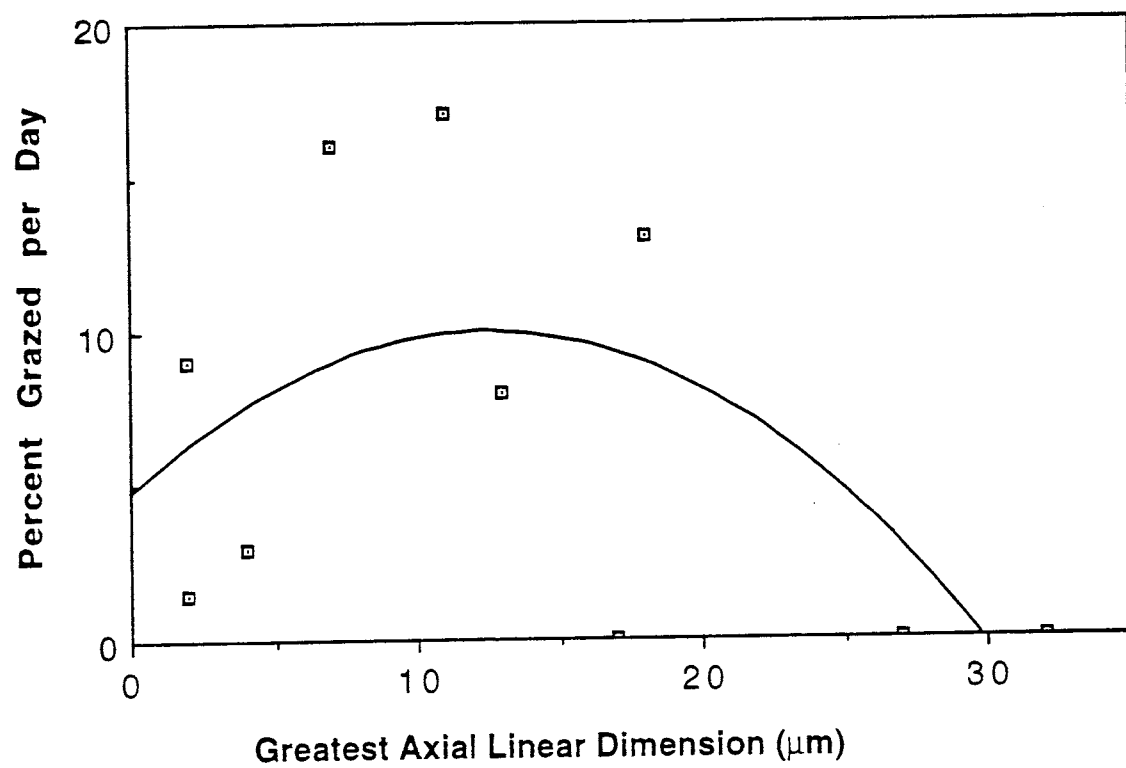


Figure 11. The relationship between species-specific phytoplankton loss rates to grazing and greatest linear axial dimension.

We did not decipher a season pattern in grazing rates. Had our analyses been confined to our "grazing experiments", we may have concluded that grazing is more substantial in spring than during summer and fall. However, rates estimated during the nutrients vs. experiment of summer 1995 were as great as those obtained during the previous spring. Apparently annual as well as seasonal variability in zooplankton and phytoplankton composition and biomass contribute to fluctuations in grazing mortality.

Finally, it should be noted that our estimates of macrozooplankton grazing on phytoplankton were essentially estimates of cladoceran grazing. The other major group of herbivorous crustaceans in lakes, calanoid copepods, were scarce during our experiments. As the vast majority of grazing studies have been done in lakes dominated by cladocerans, this aspect of our study made it more comparable with others. There is, however, an obvious need for studies in lakes dominated by calanoid copepods. Calanoid copepods are generally considered selective feeders, while cladocerans are viewed as generalists, unable to effectively differentiate between algal types (Gliwicz 1980; Richman and Dodson 1983; Strickler 1984). Our results clearly demonstrate, as do those of Lehman and Sandgren (1985), Elser and Goldman (1991) and others, that cladocerans do manage a degree of selectivity.

Grazing by Microzooplankton

Our method for estimating microzooplankton grazing on phytoplankton was not entirely successful. Two prerequisites for an accurate assessment were not met: 1) macrozooplankton were not removed from carboys with 100% efficiency (or even at equal efficiency across replicates); and 2) the phytoplankton community was not totally preserved through the sieving process (particularly large species, such as colonial diatoms, were strongly retained on sieves, while cryptophytes largely passed through; Fig. 5). The impact of macrozooplankton retention was probably to decrease weight-based estimates of clearance rates, and thus our estimates of phytoplankton losses to grazing. The reduction in phytoplankton biomass in carboys, on the other hand, may have increased microzooplankton grazing rates, as there is a general negative

relationship between food availability and clearance rates. The species affected by grazing, however, were not highly edible, suggesting that their diminished biomass may have had no effect after all.

We have chosen to report our findings in this paper despite the methodological shortcomings, first because we believe the method might work better in lakes where the ratio of macrozooplankton to microzooplankton is lower, and thus the likelihood of macrozooplankton contaminating the micrograzer treatments diminished, and second, because we believe that our data hint strongly at some relationships that should not be ignored.

Most importantly, we estimated much greater micrograzer than macrograzer clearance rates (expressed on a per gram basis) both for the phytoplankton community as a whole and for several individual divisions (cryptophytes, blue green algae, and diatoms). It was only the green algae that we found more easily cleared from suspension by macro than micrograzers. The green algal species involved (e.g., *Mougeotia*, *Oocystis*, and *Coelastrum*) were relatively large or colonial in nature, and thus presumably difficult for a small animal to handle. Rotifers and nauplii did not make up a large proportion of the zoobiomass in Lake Champlain during our experiments. However, our estimates of grazing rate indicated that the high clearance rates was made up for low biomass: micrograzing rates were consistently as great or greater than macrograzing rates. Elser and Frees (1995) recently conducted a micrograzing study in Castle Lake, CA, using the dilution method to alter grazing pressure. Like us, they concluded that micrograzing accounted for as much or more phytoplankton biomass removal per day as grazing by macrograzers (5-22% vs. 5-12% per day). Up until 1992, monitoring programs on Lake Champlain ignored rotifers. Our results, and those of Elser and Frees, indicate that the role of these animals in transferring energy and materials through foodwebs deserves more attention.

Grazing as a Mechanism of Energy and Material Flow Up Foodwebs

Our study suggested that micro and macrozooplankton together remove from 2-21% of phytoplankton biomass in the mixed layer of Lake Champlain per day. This is by coincidence the

same range of removal rates which Cyr and Pace (1992) reported for 30 phytoplankton communities in 16 northeastern U.S. lakes (their analysis was for grazers $>80\ \mu\text{m}$, and thus included the bulk of micrograzers). Thus we conclude that temporal variability in grazing rates is probably of the same order of magnitude as lake to lake variability.

The estimates of primary productivity that we obtained for Lake Champlain are within the range previously reported for large, mesotrophic lakes (Wetzel 1985). Our studies suggest that from 3-117% of the carbon fixed was funneled directly to zooplankton. The remainder probably was processed through detrital foodchains, or contributed to rising algal standing stocks. Our analysis of bacteria during the grazing experiments indicated a substantial level of bacterial productivity (equivalent to ~5-50% of primary productivity; Borchardt et al.; Section V). The range in the relative balance of phytoplankton gains and losses that we observed could easily drive seasonal changes in phytobiomass. Cyr and Pace (1993) report that, on average, aquatic herbivores remove 51% of lacustrine annual primary production, a proportion which is 3 X that effected by terrestrial herbivores. They conclude that aquatic systems have a greater grazing and a lesser detrital component than terrestrial systems.

Controls on Phytoplankton Biomass and Composition

Phytoplankton ecology has long focused on nutrients in its quest to explain phytoplankton dynamics. Phosphorus input is touted as the principal controller of standing stocks (Vollenweider 1968; Schindler 1978; Peters 1986), while both nutrient concentrations and nutrient ratios are considered major forcing functions behind species successions (e.g. Tilman et al. 1982; Sommer et al. 1986). Our nutrients versus grazers experiment supported the view that nutrients play a major role in regulating algal biomass in Lake Champlain. Combined addition of nitrogen and phosphorus to carboys fairly consistently increased overall phytoplankton biomass, even in the face of very high grazing pressure (hence ANOVA indicated a nutrient effect on chlorophyll a concentration at a significance level of 0.01). Other researchers conducting field experiments of a similar design in other lakes have obtained similar results (Lehman and Sandgren 1985; Berquist

and Carpenter 1986; Vanni 1987; Kivi et al. 1993; Proulx et al. 1996). At the species level response to nutrients is more variable. We found that some taxa, such as diatoms and green algae generally increased with fertilization (Fig. 10; Levine et al. 1997), while others, such as cryptophytes and blue green algae were unaffected or diminished in biomass. Lehman and Sandgren (1985) and Berquist and Carpenter (1986) also found negative responses to fertilization in their experiments.

We obtained further information on the nutrient status of phytoplankton in Lake Champlain by conducting enrichment experiments during the week preceding or following the grazing studies (Levine et al. 1997). These indicating a lack of nutrient limitation during May 1995, and mild P or N+P limitation in July and September 1994. During the summer-fall experiments, both N and P had to be added to enclosures to elicit a phytoplankton response. One interpretation of these results is that the phytoplankton community present during lake stratification is in equilibrium with its nutrient supply via zooplankton and bacterial recycling (Goldman 1980). In this case, neither N nor P would limit growth. Other possibilities are that light or grazing constrain community growth rates, or that P was limiting during the studies but N so scarce that increases in P availability in the absence of increased N quickly imposed N limitation.

In our earlier paper (Levine et al. 1997), we were unable to provide any evidence for light limitation in Lake Champlain; instead we noted that there was only a marginal increase in algal biomass in control enclosures incubated near the surface where one might expect pre-existing light limitations to be relieved. The light and photosynthesis data presented in this paper, however, suggests that, during May and September, Lake Champlain phytoplankton are adapted to low light intensities (as deep mixing carries them into the dark for prolonged periods), and exist in a situation where $I_{ave}:I_k < 1$. Ratios of this magnitude generally are considered indicative of light-limited growth (Fee et al. 1992). We did not estimate primary productivity in July while the lake was strongly stratified, but did note that the depths of the epilimnion and photic zone

were similar at this time (~10 m). Thus even in summer, phytoplankton in the lake must deal with low light intensities a substantial portion of the time.

The response of phytoplankton to grazers in our factorial grazers vs. nutrients experiment reflected the complexity of the zooplankton-phytoplankton relationship in lakes. ANOVA indicated only a weak relationship between chlorophyll a concentrations in carboys and grazer density ($p = 0.06$), and this relationship was *positive*. Many researchers using experimental designs similar to ours have found a similar stimulation of phytoplankton growth with grazer addition (Lehman and Sandgren 1985; Berquist and Carpenter 1986). Kivi et al. (1993) noted no chlorophyll a response to grazer manipulation, but a slight increase in primary productivity with increasing grazer density (Kivi et al. 1993). The implication of the two response types observed is that nutrient recycling by zooplankton has a greater impact on phytoplankton dynamics than does grazing mortality. Examining fertilized and unfertilized carboys separately, we confirmed that it was the nutrient-insufficient phytoplankton that were stimulated by the higher zooplankton densities (most zooplankton were carnivorous cyclopoids). For the fertilized carboys, there was a negative relationship between chlorophyll a concentration and grazer density (this estimate excluded cyclopoids).

When the relative importance of nutrients and grazers are assessed from factorial experiments such as ours it should be noted that the degree of manipulation of the two variables is frequently inequitable. Zooplankton densities are rarely raised by more than 4 or 5 fold due to concern that prey will be exhausted, whereas 10 fold increases in dissolved nutrient concentrations are routine. The increase in orthophosphate concentrations in a P limited system may be an order of magnitude higher yet. Thus there is a bias towards more discernible nutrient effects. The impact of zooplankton recycling relative to mortality effects may also be exaggerated by the typical experimental set-ups. Animals are collected over a large volume in the lake to feed in much smaller experimental systems with less phytoplankton. The N and P they excrete during the incubation may reflect their former rather than their current food supply.

We conclude from our studies that phosphorus, nitrogen, and light all influence phytoplankton growth dynamics in Lake Champlain, while grazing appears to be a significant source of mortality at least some of the time. That grazing does not always balance primary productivity indicates that losses such as sinking are probably substantial at times. Other researchers have reached similar conclusions for other lakes (e.g. Knoechel and Kalff 1975; Reynolds and Wiseman 1982).

Recently, the relationship between chlorophyll a and total phosphorus concentrations in lakes has been reexamined to consider possible impacts of grazing on biomass. Specially, data have been presorted on the basis of *Daphnia* (Mazumder 1994) or planktivorous fish (Proulx et al. 1996) densities (fish influence zooplankton abundance) before correlation analyses. The result has been substantially reduced scatter in the regressions for the subsets, and regression parameters that differ across subsets. Both findings suggest the simultaneous occurrence of bottom-up and top-down controls on phytoplankton biomass.

Advise for Future Studies

Future researchers planning to use the Lehman-Sandgren approach for measuring grazing rates may learn from the strengths and weaknesses of our experiments. First, our experiment made clear that incubation time is a critical variable in obtaining reliable estimates of phytoplankton growth rates. The 2-3 day incubations of our first three experiments were too short for the relatively low phytoplankton growth rates observed. Consequently, we obtained disappointingly low r^2 values, both for our growth rate and grazing estimates. The four day incubation of our nutrients vs. grazers experiment yielded much higher r^2 values. However, there was also a downside to the longer incubation: the zooplankton communities present in carboys had time to change. In particular, rotifer populations increased in fertilized carboys with macrograzers removed, presumably because their phytoplankton food source responded to the increased nutrient availability with higher growth rates. In addition, cladocerans were more abundant in the ambient than in the "high macrograzer" treatments, suggesting that some

carnivory may have gone on in the cyclopoid dominated HMA carboys. Since the validity of the Lehman-Sandgren approach depends on maintenance of the natural zooplankton community across treatments, this development reduced our confidence in the grazing estimates obtained. Most likely, the estimates of macrozooplankton grazing were unnaturally high: since micrograzers graze at a higher rate than macrograzers, more of them in the LMA treatment would lower the initial portion of the growth rate vs. grazer abundance curve. Another difficulty with long experiments is that the potential exists for phytoplankton populations to be grazed so heavily that zooplankton grazing rates will be effected by the lower prey density. Thus our advise to future researchers is to collect phytoplankton samples over a time course running four days or longer. Afterwards, the linear portion of the growth curve can be identified for more intensive cell counting.

Depth of water collection for the experiment is another issue that needs thought. We collected water and zooplankton principally from the mixed layer (plus 2 m into the metalimnion), thinking that phytoplankton would be confined to this region and would be too diluted if we sampled over the depth of the lake. This approach did not take into account zooplankton populations positioned deep in the lake during daylight hours, but coming to the surface to feed at night. Surveys which the NY DEC performed during the summers of 1994 and 1995 indicated that calanoid copepods were present at our sampling site, but at a depth below our net tows (C. Sandgren and S. Quinn, pers. comm.). Thus our grazing estimates may be low due to our excluding these animals.

There is also the issue of the temporal variability of grazer density in the lake resulting from vertical migrations. The grazing rates that we report are for grazer densities averaged over the depth of the mixed layer. Grazing densities are actually much greater at the surface at night, and lower during the day. Since grazing rate is influenced by food concentrations, linear averaging may not yield an appropriate measure of grazing mortality.

Although we tracked all phytoplankton species during our experiments, we recommend against doing this as the effort is too divided. It would be better to concentrate on a few species

of interest and count more cells per sample and more sample replicates. This would improve r^2 values for growth curves.

The role of cyclopoids in foodwebs is another issue deserving attention. While we include estimates of grazing based on total participation versus no participation by these animals, the actual extent to which these carnivorous animals use algal prey is unknown. Cyr and Pace (1992) have argued against substantial cyclopoid herbivory on the basis of the negative relationship that they found between phytoplankton biomass and non-cyclopoid versus the lack of any apparent relationship between phytoplankton and total zooplankton biomass. We noted the same relationships for our fertilized carboys. However, the abundance of cyclopoids during some of our experiments leaves us wondering what these animals eat, if not phytoplankton. The release of rotifers by suppression of macrozooplankton that we observed suggests that microzooplankton may be one food source. Ciliates are another possibility as their densities in samples were moderately high (Borchardt et al., Section 5).

CONCLUSIONS

The grazing experiments performed on Lake Champlain in 1994 and 1995 were troubled with a variety of methodology issues. Nevertheless, they provided valuable information on phytoplankton-grazer relationships in the lake. Grazing rates were generally low (0-20% removal per day), but sometimes balanced primary productivity. While monitoring programs on Lake Champlain have historically ignored rotifers, this study indicated that micrograzers (animals 20-200 μm across; mostly rotifers and nauplii) are as active as macrograzers in removing phytoplankton biomass from surface waters. We also found that phytoplankton vulnerability to grazing is highly taxon specific, suggesting that food web manipulations that alter grazing pressure are likely to affect species composition. Among the less vulnerable species likely to be favored by the high grazing pressure associated with biomanipulation were blue green algae. Finally, and possibly most importantly, we found that grazers are important in Lake Champlain not only because of their sequestering of phytoplankton carbon, but also because they play a significant

role in nutrient recycling. When nutrients are scarce, moderate increases in zooplankton biomass can lead to increases rather than declines in overall phytoplankton biomass.

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REFERENCES

- Arnold, D. 1971. Ingestion, assimilation, survival and reproduction by *Daphnia pulex* fed seven species of blue-green algae. *Limnol. and Oceanogr.* 16: 906-920.
- Berquist, A.M., and Carpenter, S.R. 1986. Limnetic herbivory: Effects on phytoplankton populations and primary production. *Ecology* 67: 1351-1360.
- Bogdan, K.G., and Gilbert, J.J. 1987. Quantitative comparison of food niches in some freshwater zooplankton. A multi-tracer-cell approach. *Oecologia* 72: 331-340.
- Bogdan, K.G., and McNaught, D.C. 1975. Selective feeding by *Diaptomus* and *Daphnia*. *Verh. Internat. Verein. Limnol.* 19: 2935-2942.
- Brooks, J.L., Dodson, S.I. 1965. Predation, body size, and composition of plankton. *Science* 150: 28-35.
- Carpenter, S.R., Kitchell, J.F., Hodgson, J.R. 1985. Cascading trophic level interactions and lake productivity. *BioScience* 35: 635-639.
- Cyr, H., and Pace, M.L. 1992. Grazing by zooplankton and its relationship to community structure. *Can. J. Fish. Aquat. Sci.* 49: 1455-1465.
- Cyr, H., and Pace, M.L. 1993. Magnitude and patterns of herbivory in aquatic and terrestrial ecosystems. *Nature* 361: 148-150.

- deBernardi, R.G., Giusssani, G., Pedretti, E.L. 1981. The significance of blue-green algae as food for filter-feeding zooplankton: Experimental studies of *Daphnia* spp. fed *Microcystis*. *Verh. Internat. Verein. Limnol.* 21: 477-483.
- DeMott, W.R. 1982. Feeding selectivities and relative ingestion rates of *Daphnia* and *Bosmina*. *Limnol. and Oceanogr.* 27: 518-527.
- Elser, J.J. 1992. Phytoplankton dynamics and the role of grazers in Castle Lake, California. *Ecology* 73: 887-902.
- Elser, J.J., Frees, D.L. 1995. Microconsumer grazing and sources of limiting nutrients for phytoplankton growth: Application and complications of a nutrient-deletion/dilution gradient technique. *Limnol. and Oceanogr.* 40: 1-16.
- Elser, J.J., Elser, M.M., MacKay, N.A., and Carpenter, S.R. 1988. Zooplankton-mediated transitions between N- and P-limited algal growth. *Limnol. and Oceanogr.* 33: 1-14.
- Elser, J.J., and Goldman, C.R. 1991. Zooplankton effects on phytoplankton in lakes of contrasting trophic status. *Limnol. and Oceanogr.* 36: 64-90.
- Elser, J.J., and MacKay, N.A. 1989. Experimental evaluation of effects of zooplankton biomass and size distribution on algal biomass and productivity in three nutrient-limited lakes. *Archiv. f. Hydrobiol.* 114: 481-496.
- Fee, E.J. 1990. *Computer programs for calculating in situ phytoplankton photosynthesis*. Can. Tech. Rep. Fish. Aquat. Sci. 1740: v+27.
- Fee, E.J., Shearer, J.A., BeBruyn, E.R., Schindler, E.U. 1992. Effects of lake size on phytoplankton photosynthesis *Can. J. Fish. Aquat. Sci.* 49: 2445-2459.
- Galbraith, M.G. 1967. Size selective predation of *Daphnia* by rainbow trout and yellow perch. *Trans. Amer. Fish. Soc.* 110: 1-10.
- Garnier, J., and Mourelatos, S. 1991. Contribution of grazing in phytoplankton overall losses in a shallow French lake. *Freshwater Biol.* 25: 515-523.

- Geller, W. 1984. A device for the in situ measurement of zooplankton food selection, grazing and respiration rates. *Verh. Internat. Verein. Limnol.* 22: 1425-1431.
- Gliwicz, Z.M., Hillbricht-Ilkowska 1972. Efficiency of the utilization of nanoplankton primary production by communities of filter feeding animals measured in situ *Verh. Internat. Verein. Limnol* 18: 197-203.
- Gliwicz, Z.M. 1977. Food size selection and seasonal succession of filter feeding zooplankton in an eutrophic lake. *Ekologia Polska* 25: 179-225.
- Gliwicz, Z.M. 1980. Filtering rates, food size selection, and feeding in cladocerans- another aspect of interspecific competition in filter-feeding zooplankton. In *Evolution and ecology of zooplankton communities*. ed. W.C. Kerfoot, pp. xx . Hanover, NH: Univ. Press New England. NEED PAGES
- Goldman, J.C., 1980, Physiological processes, nutrient availability and the concept of relative growth rate in marine phytoplankton ecology, *In Primary Production in the Sea*, ed. P. Falkowski, P., p. 179-194. New York: Plenum.
- Gulati, R.D., Siewertsen, K., Postema, G. 1982. The zooplankton: its community structure, food and feeding, and role in the ecosystem of Lake Vechten. *Hydrobiol.* 95: 127-163.
- Haney, J.F. 1973. An in situ examination of the grazing activities of natural zooplankton communities. *Archiv. f. Hydrobiol.* 114: 481-496.
- Havens, K.E. 1991. The importance of rotiferan and crustacean zooplankton as grazers of algal productivity in a freshwater estuary. *Archiv. f. Hydrobiol.* 122: 1-22.
- Hrbacek, J., Dvorakova, M., Korinek, V., and Prochazkova, L. 1961. Demonstration of the effect of the fish stock on the species composition of zooplankton and the intensity of metabolism of the whole plankton association. *Verh. Internat. Verein. Limnol.* XIV: 192-195.

- Hutchinson, B.P. 1971. The effect of fish predation on the zooplankton of ten Adirondack lakes with particular reference to the alewife, *Alosa pseudoharengus*. *Trans. Amer. Fish. Soc.* 110: 325-335.
- Infante, A., Abella, S. 1985. Inhibition of *Daphnia* by *Oscillatoria* in Lake Washington. *Limnol. and Oceanogr.* 30: 1046-1052.
- Infante, A.D. 1978. Natural food of herbivorous zooplankton of Lake Valencia (Venezuela). *Archiv. f. Hydrobiol* 82: 347-358.
- Jarvis, A.C. 1986. Zooplankton community grazing in a hypertrophic lake (Hartbeespoort Dam, South Africa). *J. Plankton Res.* 8: 1065-1078.
- Jarvis, A.C., Hart, R.C., Combrink, S. 1988. Cladoceran filtration rate-body length relations: model improvements developed for a *Microcystis*-dominated hypertrophic reservoir. *J. Plankton Res.* 10: 115-131.
- Kivi, K., Kaitala, S., Kuosa, H., Kuparinen, J., Leskinen, E., Lignell, R., Marcussen, B., and Tamminen, T. 1993. Nutrient limitation and grazing control of the Baltic plankton community during annual succession. *Limnol. and Oceanogr.* 38: 893-905.
- Knisely, K., and Geller, W. 1986. Selective feeding of four zooplankton species on natural lake phytoplankton. *Oecologia* 69: 86-94.
- Knoechel, R., Kalff, J. 1975. Algal sedimentation: the cause of a blue-green diatom succession. *Verh. Internat. Verein. Limnol* 19: 745-754.
- Lair, N. 1991. Comparative grazing activities of the rotifer and crustacean communities in a eutrophic lake. *Verh. Internat. Verein. Limnol.* 24: 924-927.
- Lampert, W. 1981. Inhibitory and toxic effects of the toxic *Microcystis aeruginosa*. *Int. Rev. ges. Hydrobiol.* 66: 285-296.
- Lampert, W., Fleckner, W., Rai, H., and Taylor, B.E. 1986. Phytoplankton control by grazing zooplankton: A study on the spring clear-water phase *Limnol. and Oceanogr.* 31: 478-490.

- Landry, M.R., and Hassett, R.P. 1982. Estimating the grazing impact of marine micro-zooplankton. *Marine Biol.* 67: 283-288.
- Larow, E.J., McNaught, D.C. 1978. Systems and organismal aspects of phosphorus remineralization. *Hydrobiol.* 59: 151-154.
- Lehman, J.T. 1980. Release and cycling of nutrients between planktonic algae and herbivores. *Limnol. and Oceanogr.* 25: 620-632.
- Lehman, J.T., and Sandgren, C.D. 1985. Species-specific rates of growth and grazing loss among freshwater algae. *Limnol. and Oceanogr.* 30: 34-46.
- Levine, S.N., Shambaugh, A.d., Pomeroy, S.E., Braner, M 1997. Phosphorus, nitrogen, and silica as controls on phytoplankton biomass and species composition in Lake Champlain (USA-Canada). *J. Great Lakes Res.* 23: 133-150.
- Lorenzen, C.J. 1967. Determination of chlorophyll and pheopigments: Spectrophotometric equations. *Limnol. and Oceanogr.* 12: 343-346.
- Mazumder, A. 1994. Phosphorus-chlorophyll relationships under contrasting herbivore and thermal stratification: predictions and patterns *Can. J. Fish. Aquat. Sci.* 51: 390-400.
- McCauley, E. 1984. The estimation of the abundance and biomass of zooplankton in samples. *In A manual of methods for the assessment of secondary productivity in freshwaters.* eds. J. Downing and F. Rigler, F., pp. 228-265. Blackwell Scientific.
- TOWN
- McIntosh, A., Brown, E., Duchovnay, A., Shambaugh, A., Williams, A., 1993. 1992 *Lake Champlain Biomonitoring Program.* Burlington, VT: , University of Vermont.. Vermont Water Resources and Lake Studies Center
- Mills, E.L., Forney, J.L., Wagner, K.J. 1987. Fish predation and its cascading effect on the Oneida Lake food chain. *In Predation: direct and indirect impacts on aquatic communities,* eds. pp. xx. Hanover, NH: University Press of New England.

Myer, G.E., Gruendling, G.K., 1979, *Limnology of Lake Champlain*. Burlington, VT:
New England River Basin Commission.

Peters, R.H. 1986. The role of prediction in limnology. *Limnol. and Oceanogr.* 31: 1160-1166.

Peters, R.H., and Downing, J.A. 1984. Empirical analysis of zooplankton filtering and feeding rates. *Limnol. and Oceanogr.* 29: 763-784.

Porter, K.G. 1973. Selective grazing and differential digestion of algae by zooplankton. *Nature* 224.

Porter, K.G. 1977. The plant-animal interface in freshwater ecosystems. *American Scientist* 65: 159-170.

PORTER AND ORCUTT 1980 MISSING

Proulx, M., F.R. Pick, A. Mazumder, P.B. Hamilton, and D.R.S. Lean. 1996. Effects of nutrients and planktivorous fish on the phytoplankton of shallow and deep aquatic systems. *Ecology* 77: 1556-1572.

REINERTSEN ET AL 1990

Reynolds, C.S., Wiseman, S.W. 1982. Sinking losses of phytoplankton in closed limnetic systems. *J. Plankton Res.* 4: 489-522.

Reynolds, C.S. 1984. *The ecology of freshwater phytoplankton*. Cambridge: Cambridge University Press.

Richman, S., and Dodson, S.I. 1983. The effect of food quality on feeding and respiration by *Daphnia* and *Diaptomus*. *Limnol. and Oceanogr.* 28: 948-956.

Sarnelle, O. 1992. Contrasting effects of *Daphnia* on ratios of nitrogen to phosphorus in a eutrophic, hard-water lake. *Limnol. and Oceanogr.* 37: 1527-1542.

Sartory, D.P., and Grobbelaar, J.U. 1984. Extraction of chlorophyll a from freshwater phytoplankton for spectrophotometric analysis. *Hydrobiol.* 114: 177-187.

- Scavia, D., Lang, G.A., Kitchell, J.F. 1988. Dynamics of Lake Michigan plankton: a model evaluation of nutrient loading, competition, and predation *Can. J. Fish. Aquat. Sci.* 45: 165-177.
- Schindler, D.W. 1978. Factors regulating phytoplankton production and standing crop in the world's lakes. *Limnol. and Oceanogr.* 23: 478-486.
- Schoenberg, S.A., and Carlson, R.F. 1984. Direct and indirect effects of zooplankton grazing on phytoplankton in a hypereutrophic lake. *Oikos* 42: 291-302.
- Shapiro, J., Lamarra, V., Lynch, M. 1975. Biomanipulation: an ecosystem approach to lake restoration. In *Proceedings of a symposium on water quality management through biological control*, eds. P.L Brezonik and J.L. Fox, pp. xx. Washington, DC: U.S. Environmental Protection Agency. PAGES
- Shapiro, J., Forsberg, B., Lamarra, V., Lindmark, G., Lynch, M., Smeltzer, E., Zoto, G., 1982, *Experiments and experiences in biomanipulation- studies of biological ways to reduce algal abundance and eliminate blue-greens*. Corvallis, OR, Corvallis Environmental Research Laboratory.
- Shapiro, J., and Wright, D.J. 1984. Lake restoration by biomanipulation: Round Lake, Minnesota, the first two years. *Freshwater Biol.* 14: 371-383.
- Sommer, U., Gliwicz, Z.M., Lampert, W., and Duncan, A. 1986. The PEG-model of seasonal succession of planktonic events in fresh waters. *Archiv. f. Hydrobiol* 106: 433-471.
- Stenson, J.A.E., Bohlin, T., Henrikson, L., Nilsson, B.I., Nyman, H.G., Oscarson, H.G., Larson, P. 1978. Effects of fish removal from a small lake. *Verh. Internat. Verein. Limnol* 20: 794-801.
- Sterner, R.W. 1989. The role of grazers in phytoplankton succession. In *Plankton ecology: succession in plankton communities*, ed. U. Sommer, pp. 107-170. Berlin: Springer-Verlag.

- Sterner, R.W. 1990. The ratio of nitrogen to phosphorus resupplied by herbivores: Zooplankton and the algal competitive arena. *Amer. Nat.* 136: 209-229.
- Svensson, J.-E., and Stenson, J.A.E. 1991. Herbivoran impact on phytoplankton community structure. *Hydrobiol.* 226: 71-80.
- Tilman, D., Kilham, S.S., and Kilham, P. 1982. Phytoplankton community ecology: The role of limiting nutrients. *Ann. Rev. Ecol. Sys.* 13: 349-372.
- Tilzer, M.M. 1984. Estimation of phytoplankton loss rates from daily photosynthetic rates and observed biomass changes in Lake Constance *J. Plankton Res.* 6: 309-324.
- Vanderploeg, H.A. 1981. Seasonal particle-size selection by *Diaptomus sicilis* in offshore Lake Michigan. *Can. J. Fish. Aquat. Sci* 38: 504-517.
- Vanderploeg, H.A., Paffenhofer, G.A., Liebig, J.R. 1988. Diaptomus vs. net phytoplankton: effects of algal size and morphology on selectivity of a behaviorally flexible, omnivorous copepod. *Bull. Mar. Sci.* 43: 377-394.
- Vollenweider, R.A., 1968, *The scientific basis of lake stream eutrophication, with particular reference to phosphorus and nitrogen as eutrophication factors*. Paris: OECD.
- Wetzel, R.G. 1983. *Limnology*. Philadelphia: Sauders College Publishing.
- Wetzel, R.G., Likens, G.E. 1991. *Limnological analyses*. New York: Springer-Verlag.

Importance of Grazers in Controlling Bacterioplankton and Heterotrophic Protozoa in Lake Champlain

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INTRODUCTION

Lake Champlain includes a diverse assemblage of organisms, most of them microscopic, connected in a complex food web. All too often complex interactions among organisms become manifest in hindsight, when human perturbations to a lake's food web are translated into undesirable impacts on species of special interest, such as game fishes, or nuisance algae. Phosphorus reduction, fish stocking and lamprey control are management programs currently underway in Lake Champlain that may have unintended consequences on the lake's biota and chemistry. Microbes (bacteria, protozoa, algae) are the foundation of the lake food web. It is essential to the successful and long-term management of Lake Champlain to understand the lake's microscopic world.

The early concept of energy flow in a lake was as a linear food chain, comprised of phytoplankton, zooplankton, planktivorous fish, and piscivores. Bacteria and heterotrophic protozoa were ignored. Recent studies, however, have shown that energy transfer is not so linear, and carbon excreted by algae (5-50% of the total C fixed) is used by bacteria, some of which are in turn consumed by protozoa (Pomeroy 1974, Azam et al. 1983). This energy path has been termed the microbial loop (Azam et al. 1983), and its contribution to higher trophic levels remains controversial.

Any management effort or perturbation that suppresses or promotes the microbial loop may alter the portion of algal production available to a fishery. For example, nutrient ratios that favor algal taxa with high organic matter excretion rates may enhance microbial production. Or the presence of planktivorous fish may reduce the proportion of bacterial production passed on to zooplankton and increase the number of protists (Riemann 1985). Knowledge of the microbial food web in Lake Champlain will provide the framework for modeling and prediction of the consequences of various perturbations or management strategies.

The specific objectives of this study were:

1. To measure the rates at which bacteria and protozoa are grazed by zooplankton.
2. To measure bacterial productivity in the Main Lake.
3. To evaluate the relative importance of carbon flow through a portion of the microbial loop (from bacteria to protozoa) versus up the classic food chain (from algae to zooplankton) in the lake.
4. To evaluate the short-term changes in food web structure when nutrient concentrations or grazer densities are altered.

METHODS

Site Description

Lake Champlain is a large deep lake (170 km long; 20 km maximum width; 1,130 km² surface area; maximum depth 122 m; mean depth 23 m), bordering the states of Vermont and New York and the province of Quebec. It empties north through the Richelieu River, a tributary of the St. Lawrence River, and is connected to the south to the Hudson River via the Lake

Champlain Canal. Numerous islands, sills, peninsulas and causeways divide the lake into partially isolated sub-basins. Our study took place in the largest of these, known as "Main Lake."

Main Lake is generally dimictic (complete ice cover occurs for at most two months) and mesotrophic-oligotrophic. Physiological indicators of P status suggest that phytoplankton in the lake alternate between P sufficiency and deficiency (Levine et al. 1997). P appears to be the principal limiting nutrient, N may be co-limiting at times (Levine et al. 1997). Our sampling site was at 44° 27' 36" N, 73° 17' 39" W, about 1 km northwest of Juniper Island, at the broadest expanse of the lake.

Grazing Experiments

Three experiments were conducted (July 25-27, 1994; September 27-29, 1994; May 8-11, 1995) to assess rates of bacterivory by all grazers and grazing rates on heterotrophic nano-protozoa (HNP) and cryptomonads by macrozooplankton. Grazer densities were manipulated in experimental carboys (10 L, polyethylene) and the change in prey abundance in each carboy was monitored for two to three days. Prey density was log transformed and regressed on time to yield the specific growth rate. Prey specific growth was then regressed on grazer biomass; the resulting equations have slopes equal to the clearance rates of the grazer on the prey, and the y-intercepts equal the growth rate of the prey in the absence of the grazers (Lehman and Sandgren 1985).

An experiment began by collecting 200 L water from the study site into a 240 L polypropylene tank. Water was collected from the epilimnion and 2 m into the metalimnion with an 8 L Alpha bottle, deployed at 1 m intervals downward and 5 m intervals upward through the water column. In the May 95 experiment, when the lake mixed to the bottom, we sampled to 20 m, the same depth as the September 94 experiment. Epilimnion depth was determined at the time of the experiment from a CTD cast (Seacat Profiler V1.8b). Care was taken to prevent exposing organisms to sunlight at surface intensities. Macrozooplankton were concentrated for the high density zooplankton treatment by hauling a 202 μm -mesh Wisconsin net (collar off; mouth diameter 0.5 m, mason jar attached) up through the same depth interval two times and releasing the animals into 30 L of lake water (release was underwater to minimize air trapping under cladoceran carapaces).

Grazer treatment levels were established as depicted in Figure 1. All levels were duplicated. The "ambient macrograzer" carboys received unaltered lake water. The "high macrograzer" treatment had a target macrozooplankton density of 4 fold greater than ambient and was created by adding 300-500 mL of the macrozooplankton concentrate to carboys filled with lake water. The "low macrograzer" treatment was created by filling carboys with lake water passed through either a 202 μm plankton net (July) or a 220 μm sieve (September and May). The "low macrograzer" carboys also served as the "ambient micrograzer" treatment. Sievate from the 202 μm net or 220 μm sieve (macrozooplankton removed) was then passed through a 20 μm sieve and the retained grazers were added back to a carboy filled with the 202 (or 220) μm sievate to create the "high micrograzer" treatment. Forty liters was sieved and concentrated in

10 L to increase micrograzers 4 fold above ambient. The “low micrograzer” treatment was created by filling carboys with 20 µm sievate. This treatment also served as the “ambient nanograzer” treatment. The last step of the manipulation was to filter 10 L of 20 µm sievate through 47 mm diameter GF/F Whatman glass fiber filters to remove nanograzers. Filters were changed after 1 L had been filtered. Nanograzers could not be recovered and added back to create a “high nanograzer” treatment, and thus, there were two instead of three nanograzer treatment levels.

The grazer size group and the taxa that were altered were as follows: Macrozooplankton = calanoid copepods, cyclopoid copepods, cladocerans and the large rotifer *Asplanchna*. Micrograzers = rotifers (other than *Asplanchna*), copepod nauplii and *Cryptomonas* sp. Nanograzers = heterotrophic protozoa < 20 µm and *Chroomonas* sp. The manipulation for macrozooplankton did not alter micrograzer densities, and the micrograzer manipulation did not alter nanograzer densities.

Finally, 0.3 µM KH_2PO_4 , 5 µM NH_4Cl and 5.7 µM dextrose ($\text{C}_6\text{H}_{12}\text{O}_6$) (final concentrations) were added to each carboy to eliminate the gradient in nutrient regeneration caused by the gradient in grazer densities among carboys. Without adding nutrients, prey may not respond to a decrease in grazing pressure and the grazing rate may be underestimated (Lehman and Sandgren 1985). On the other hand, adding nutrients may bias upward the estimate of prey specific growth rate free of grazing mortality (y intercept). To measure bacterial production in the lake without the nutrient enrichment effect, we established two additional “low nanograzer” carboys, but did not add nutrients. To assess the potential impact of sieving on

dissolved nutrient concentrations, we collected samples for DOC, DON, and TDP analysis from whole water, the 202 (or 220) μm sievate and the 20 μm sievate.

The carboys were incubated just outside the breakwater of Burlington Harbor for two to three days (44, 48, and 64 hours in July 94, September 94 and May 95 experiments, respectively) suspended from anchored floating frames at a depth of 1.5 m. Bacteria and nanograzers were sampled at the beginning of an incubation and three to four times thereafter at 6 to 24 hour intervals. Macrozooplankton, rotifers and nauplii were sampled at the end of an incubation.

Macrograzer and Nutrient Control of Bacterioplankton and HNP

We conducted a 3x2 factorial experiment from July 31 to August 4, 1995 (August 95 experiment) to gauge the role of macrozooplankton and nutrients as controls on the abundance of bacterioplankton and HNP. Water was collected (300 L) from 0 to 15 m in 1 m and 3 m intervals in the same manner and at the same site as for the grazing experiments. Macrozooplankton were the only grazer group manipulated; "high", "ambient", and "low" macrograzer densities were created as described previously (Figure 1). Half the carboys at each grazer level were enriched with C, N, and P at the same concentrations used in the grazing experiments, while the other half were left unfertilized. In this way six treatments (high, ambient and low grazer density, each with or without nutrient amendment) were created in triplicate. Carboys were incubated for four days (92 hours) using the same site, frames, and depth as for the grazing experiments.

Response variables were the specific growth rates of bacteria and HNP, obtained by regressing the log transformed numerical abundance of these prey groups on time. Three

replicate samples for bacteria and two duplicate samples for HNP were withdrawn from each carboy at the beginning of the experiment and 23 and 92 hours later. Macrozooplankton, rotifers, and nauplii were collected from the carboys at the end of the experiment. The density of these grazers at the study site was estimated from two 10 L samples taken from the large tank containing the 300 L of ambient lake water. Data were analyzed as a balanced two-way ANOVA, including the grazer x nutrient interaction term, using SAS (v. 6.11).

Grazer and Prey Enumeration

Macrozooplankton, rotifers and nauplii were collected from carboys by pouring the contents through a 63 μm (July) or 20 μm (September and May) sieve, gently rinsing the organisms into a 500 mL bottle, anaesthetizing them with carbonated water and adding an equal volume of 10% formalin/sucrose (80 g/L) with Rose Bengal for staining. Zooplankton were enumerated and measured with a dissecting scope. Cladoceran and copepod biomasses were estimated from biomass-length relationships (McCauley, 1984), and rotifer biomass was estimated from cell dimensions and geometry (Ruttner-Kolisko, 1977). *Cryptomonas* sp.(average maximum length in Lake Champlain = 23 μm) was enumerated from the nanograzer samples.

Nanograzers were sampled by three 30 mL aliquots per carboy per time period and fixed by adding sequentially 15 μL alkaline Lugol's solution, 900 μL nonbuffered formalin (3% final concentration) and 20 μL sodium thiosulfate to clear the iodine stain. Samples were refrigerated until filtered, < 24 hours after collection. Nanograzers were filtered onto 25 mm diameter,

0.8 μm pore size, preblackened filters (Nuclepore) using no more than 100 mm Hg vacuum. Filtration volume was 20 mL, except for samples from the "high micrograzer" carboys in the May 95 experiment, which was 10 mL. One mL DAPI (50 $\mu\text{g/mL}$) was applied to a filter while on the pedestal, allowed to stand for 5 minutes, and then rinsed with filtered distilled water. Filters were mounted to glass slides with immersion oil (Cargille Type A) and a coverglass sealed around the edge with nail polish. Slides were stored frozen (-20°C). Nanograzers were enumerated with an epifluorescence microscope (Nikon Optiphot, filter cube UV 2A, Ex 330-380, DM 400, BA 420), viewing 20 to 40 randomly selected fields per filter, and grouped as follows: HNP $< 5 \mu\text{m}$; HNP 5-12 μm ; HNP 12-20 μm ; *Chroomonas* sp; *Cryptomonas* sp. (placed in the micrograzer category). Large ciliates were rare, probably due to the small sample volume.

Bacteria were sampled by three 40 mL aliquots per carboy per time period, fixed with nonbuffered formalin (3% final concentration) and kept on ice or refrigerated until enumeration. Flow cytometry was used to enumerate bacterioplankton. Flow cytometry has many advantages over microscopic observations: 1) population estimates are more accurate as all bacteria are counted, including those too small or faintly fluorescent to be resolved by microscopy; 2) reproducibility is $\pm 5\%$ as opposed to $\pm 20\%$ for microscopic counts; 3) samples can be counted quickly (approximately 5 min/sample) with little fatigue to the operator; 4) photosynthetic nanoflagellates the same size as bacteria can be discerned and not mistakenly counted as bacteria; and 5) characteristics which segregate subpopulations within the bacterial community

such as size, DNA content, and RNA content can be obtained for each cell (Button and Robertson 1993).

We used a Coulter flow cytometer fitted with an EPICS-C 5W argon laser, three photomultiplier tubes for light side scatter and red and green fluorescence, a high sensitivity photodiode for forward light scatter, 3-decade logarithmic circuitry, and a quartz flow cell with a 76 μm orifice. Samples were pre-filtered (3 μm pore size) to remove debris, and the bacteria stained with propidium iodide for a combined DNA/RNA signal. An internal standard of 1 μm green-fluorescent beads was simultaneously counted to determine sample volume and to check on instrument stability and sensitivity. Bacteria were discriminated from small protozoa and algae, inert particles and the fluorescent beads on the basis of forward light scatter and red fluorescence. Counting was halted at 10,000 bacteria. Repeated counts of the same sample yielded a coefficient of variation of 1.4% ($n=5$).

Flow cytometry counts were periodically confirmed with epifluorescent microscopic counts; bacteria were stained with propidium iodide and filtered onto a 0.2 μm pore size pre-blackened filter (Nuclepore). Counts were conducted at a magnification of 1000x with a Nikon Optiphot microscope (filter cube G2-A, Ex 510-560, DM 575, BA 590).

RESULTS AND DISCUSSION

Lake conditions. Three experiments were conducted under summer stratification conditions, and one experiment was conducted when the lake was fully mixed in the spring. Epilimnion depths ranged from 8 to 18 m and the surface water temperature, at which grazing

rates were measured, ranged from 5 to 22°C (Figure 1). Lake Champlain is a temperate dimictic lake, and the thermal and oxygen profiles during the experiments were typical for the lake.

Ideally, with additional funds, grazing experiments would have been replicated under the same lake conditions for more than one year and additional experiments would have been conducted at other times of the year, notably during autumn mixing and when the lake is covered with ice. The community composition of the lake is likely different at these times compared to the spring and summer. The grazing data presented below, therefore, give several snapshots of trophic interactions in the lake. Additional experiments would complete the dynamic picture.

Bacterial abundance and productivity. Bacterial numerical abundance in the epilimnion varied approximately two-fold from 1.14×10^9 cells/L in the spring of 1995 to more than 2×10^9 cells/L in the summers of 1994 and 1995 (Table 1). Bacterial abundance in lakes is directly related to nutrient levels. Although, bacterioplankton in Lake Champlain have not been enumerated over the complete range of nutrient levels that vary seasonally or by lake site, it is likely that the general model which describes bacterioplankton abundance as a function of phosphorus concentration is also applicable to Lake Champlain.

Bacterial productivity could be estimated in the first three experiments; in the fourth experiment only macrozooplankton were manipulated and the relationship between macrozooplankton biomass and bacterial specific growth was positive, precluding an estimate of productivity. Bacterial specific growth rates during the three experiments ranged from 0.15 to 0.57 d^{-1} (Table 1), or, expressed in terms of generation times, from 4.6 to 1.2 days. Assuming that the number of bacterioplankton at the time of the experiments was in equilibrium, then

productivity ranged from 1.70×10^8 to 11.71^8 cells/L/d. At these rates the entire bacterial population would turn over every 1.8 to 6.7 days. The slower growth rate and longer turnover time occurred during the May 95 experiment, when the water temperature was coldest, between 4 and 5°C. Interestingly, for the three experiment dates, specific growth rate corresponded to lake temperature. It may be possible for future modeling efforts of trophic interactions in Lake Champlain to use a temperature-dependent growth model to predict bacterioplankton growth rates.

While the estimates of specific growth in the carboys are likely accurate ($p = 0.001$, 0.16 and 0.07, Table 1), it is important to keep the caveats of the method in mind and how the estimates may differ from what is actually occurring in the lake. Because bacterial growth was negligible in the carboys where all grazers had been removed and nutrients had not been added (data not shown), the productivity estimates were derived from carboys with added nutrients. Hence, the productivity estimates may be biased upward. The lack of growth in carboys without grazers or nutrient augmentation suggest tight coupling between nutrient regeneration by grazers and bacterial growth. Insofar that the added nutrients represent grazer nutrient regeneration, the productivity estimates will accurately depict bacterioplankton growth in the lake.

On the other hand, productivity during the July 94 experiment may be greater than estimated. The intercept of bacterial specific growth regressed on grazer biomass indicates the growth rate free of the mortality losses due to the grazer group that was manipulated. In the July 94 experiment, the smallest grazer group that was manipulated and yielded results was the micrograzers. Mortality from nanograzers was unaccounted, and may have had a net effect of

decreasing the productivity estimate. The bias may be small given the low nanograzer biomass present in July 94 (Figure 5).

Caveats and biases notwithstanding, these are the first bacterioplankton productivity estimates for Lake Champlain and they are within normal limits for bacterial growth. There is nothing to suggest, in the abundance or productivity data, that the bacterioplankton dynamics in Lake Champlain are anomalous.

Grazer abundance. The abundance of invertebrate and protozoan grazers and the relative proportion of each size and taxonomic group varied by season. Members of the grazer size categories, macro, micro, and nano, are listed in Figures 2-5. Total grazer biomass was lowest during the July 94 experiment at 100.3 μg dry wt/L and highest during the September 94 experiment at 1267.4 μg dry wt/L. On all experiment dates macrozooplankton constituted the greatest proportion of grazer biomass, as much as 8-fold more than the next largest group (Figure 2). Micrograzers were the second most abundant group in terms of biomass, except for the July experiment when nanograzers ranked second.

The dominant macrozooplankton were either cladocerans or cyclopoid copepods (Figure 3). Cladocerans composed 96% of the biomass in July, but were more abundant in September, increasing to 265.3 from 73.7 during the two month period between experiments. Three cladoceran species are common in the lake: *Daphnia galeata mendota*, *Daphnia retrocurva*, and *Bosmina longirostris*. Cyclopoids (mostly *Mesocyclops edax* and *Diacyclops bicuspidatus thomasi*) had the highest biomass of any of the groups reaching 758.1 and 537.3 μg dry wt/L, representing 73% and 72% of macrozooplankton biomass at the time of the September

and May experiments, respectively. Calanoid copepods (e.g., *Diaptomus minutus* and *Diaptomus silicis*) had their greatest abundance (153.9 µg dry wt/L) and greatest relative proportion (29%) during the May experiment. The large rotifer *Asplanchna* was a minor component of the macrozooplankton during all experiments.

Micrograzer biomass was lowest in July (5.1 µg dry wt/L) and highest in September (159.8 µg dry wt/L) (Figure 4). The July value is likely underestimated because, unlike the other grazer biomass samples which were taken from the lake at the beginning of the experiment, the July micrograzer samples were drawn from the ambient treatment carboys at the end of the experiment. This is because the initial lake samples were sieved inappropriately for enumerating micrograzers. Micrograzer abundance at the end of the experiment may have been reduced from macrograzer predation.

Each micrograzer group predominated at one time during the grazing experiments: copepod nauplii in July (97% of total), *Cryptomonas* in September (63% of total), and rotifers in May (48% of total) (Figure 4). During the grazing/nutrient experiment in August 95, rotifers were the dominant micrograzer (74 µg dry wt/L), comprising 68% of the total micrograzer biomass. Common rotifer taxa in the lake are *Keratella cochlearis*, *Polyarthra major*, *Kellicottia longispina* and *Notholca* sp.).

Nanograzer biomass was low in the July 94 experiment (18.6 µg dry wt/L), similar to the other groups at this time, and in the August 95 experiment (Figure 5). Nanograzer biomass during the September and May experiments was similar, 69 µg dry wt/L. *Chroomonas* sp. and

heterotrophic protozoa in the 5-12 μm size category composed most of the biomass in this grazer group.

Bacterivory. In each of the three experiments designed to measure grazing rates, bacterivory could be measured, although statistical associations were at times weak (Table 1). Our view is that with sufficient replication, marginally significant associations would likely have met the standard definition of statistical significance ($p < 0.05$), as demonstrated by the number of significant associations found in the two-way ANOVA experiment described below which had three instead of two replicates per treatment. Following a standard definition of significance would have excluded some potentially revealing information. Instead, we use an r^2 value of 0.20 as the threshold for reporting associations. All p and r^2 values are reported for the reader to interpret the likelihood that a rate or association is correct.

An additional consideration to keep in mind is that the experimental design adopted for this study was intended to yield grazing information for specific size categories of grazers, not specific grazer taxa. Grazer densities within a specific size category are correlated, for example, manipulating the density of rotifers also altered the density of nauplii. Thus it is not possible to attribute clearance rates to specific taxa; statistical associations and the rates derived thereof can only be attributed to the grazer size group. Some inferences on specific taxa can be drawn from the relative abundances of the taxa within a group, i.e., assume the dominant group yields the largest effect. In contrast, clearance rates on specific prey taxa can be estimated because prey taxa are not correlated by the experimental manipulation.

Bacterivory could be measured once for each grazer size category on different experiment dates: macrozooplankton, July 94; nanograzers, September 94; and micrograzers, May 95 (Table 1). Grazer clearance rates on bacterioplankton (mL/ μ g dry wt/d) were 1.1 for macrozooplankton, 0.2 for micrograzers, and 4.9 for nanograzers.

Carbon fluxes from bacteria to grazers can be estimated by assuming that bacteria have a cell density of 1.1×10^{-12} g/ μ m³, an 80% water content and a 50 % carbon content (Luria 1960). Bacterioplankton were not sized in our study, but a reasonable volume estimate for aquatic bacteria is 0.13 μ m³/cell (Bott and Kaplan 1985). Thus the bacterial dry weight would be 2.86×10^{-14} g or 1.43×10^{-14} g C per cell. Multiplying the clearance rate by bacterial density, the carbon conversion factor, and the density of grazers exhibiting bacterivory on that date yields carbon fluxes (mg C/L/d) of 2.5 for macrozooplankton (July 94), 6.6 for nanograzers (September 94) and 0.4 for micrograzers (May 95).

Macrozooplankton had measurable bacterivory rates when cladocerans were the dominant grazer; otherwise macrozooplankton had a positive effect on bacterial growth (Table 2). Cladocerans like *Daphnia* and *Bosmina* are capable of ingesting bacteria, and indeed, among the three grazer size categories and experiment dates, the macrograzers in the July 94 experiment had the strongest relationship between grazer density and bacterial growth, when cladocerans were dominant.

Micrograzers had a statistically significant positive relationship in the September 94 experiment when *Cryptomonas* was dominant. There is some evidence that *Cryptomonas* can phagocytize bacteria, but in the September 94 experiment, there was no evidence for bacterivory

by *Cryptomonas*. Bacterivory by micrograzers was detected when rotifers were abundant (May 95 experiment), consistent with the bacterivorous feeding habit of many planktonic rotifers.

Heterotrophic protozoa, a component of the nanograzer group in this study, are the classic bacterivores in the microbial loop. Their ability to rapidly and efficiently ingest bacteria is well documented. It should have been possible to measure bacterivory by nanograzers in each experiment. The fact that we could detect bacterivory only in the September 94 experiment, when the mixotroph *Chroomonas* was dominant, suggests that other factors controlling or masking bacterivory by heterotrophic protozoa in the July 94 and May 95 experiments were operating. Heterotrophic protozoa were negatively affected by either macrozooplankton or micrograzers in all three grazing experiments (Table 6).

At times macrozooplankton and micrograzers had a positive effect on bacterial specific growth (Table 2). This positive effect could be due to two phenomena, acting separately or in combination: 1) a direct effect from enhanced nutrient regeneration via fecal excretion, or 2) an indirect effect from large grazers, reducing the numbers of small bacterivorous grazers via predation or interference competition. There is evidence to suggest that both phenomena occurred. Bacterial growth was negligible in carboys with grazers excluded, and nutrients were not added, suggesting that bacteria were dependent solely on nutrient regeneration for growth, and implying that more nutrient regeneration would mean more growth. Evidence for the indirect effect was seen in the September 94 and August 95 experiments (see below) when macrozooplankton simultaneously had a negative effect on heterotrophic protozoa and a positive effect on bacteria (Tables 6 and 7).

In summary, bacterivory could be measured when a grazer size group was dominated by a taxon known to be bacterivorous, specifically cladocerans or rotifers. Carbon fluxes to grazer size categories were on the order of milligrams carbon per liter per day. Nonbacterivorous grazers can have a significant enhancement effect on bacterial growth via nutrient regeneration, or indirectly via predation or interference competition on smaller bacterivorous grazers.

Macrozooplankton feeding on heterotrophic protozoa and cryptomonads. In the July 94 experiment, heterotrophic protozoa were cleared from the water by macrozooplankton at 3.5 mL/ μ g dry wt/ d. Cladocerans dominated the macrozooplankton at the time of the experiment. Although the calculated clearance rate is weak statistically ($p=0.27$, $r^2 = 0.29$), it is similar to clearance rates obtained for a cladoceran-dominated macrozooplankton community feeding on heterotrophic protozoa in Lake Michigan (Carrick et al, 1991). No association could be measured between macrozooplankton and cryptomonads (Table 3), probably because the biomass of *Cryptomonas* and *Chroomonas* in July 94 was so low, the lowest measured in the study (Figures 3 and 4).

In the September 94 experiment macrozooplankton fed on both heterotrophic protozoa and cryptomonads. Rates were low, 0.02 to 0.1 mL/ μ g dry wt/d, but most of the associations tested were highly significant statistically (Table 3). Measurable clearance rates could be obtained, probably because of the abundance of the grazer and prey communities. Macrozooplankton and cryptomonad biomasses were the highest measured of the three grazing experiments, 1038 and 147 μ g dry wt/L, respectively. Cyclopoid copepods were the dominant

zooplankter, but cladoceran biomass was almost four times greater than the July 94 experiment (Figure 2).

Only one weak negative association was measured in the May experiment (Table 3); one was very weakly positive, and the rest were nonsignificant. In other words, macrozooplankton were not measurably feeding on cryptomonads and heterotrophic protozoa. These prey food items were present and available. Notable differences in the prey and grazer communities between this and the other two experiments were the high biomass of calanoid copepods and the low biomass of cladocerans.

In summary, macrozooplankton clearance rates on heterotrophic protozoa ranged from 0.02 to 4.2 mL/ μ g dry wt/d and on cryptomonads from 0.02 to 0.1 mL/ μ g dry wt/d (Table 3). It is more probable that the lower rates, on the order of hundredths to tenths of a milliliter per day, represent the true clearance rates, given the high r^2 and low p values obtained from these regressions. More generally, it can be concluded for these three experiments that macrozooplankton had either a negative or zero effect on heterotrophic protozoa and cryptomonads (only one positive association was detected), and the effect varied by experiment date. No specific prey category (e.g., heterotrophs $<5\ \mu$ m) were grazed consistently across the three experiments. The variable effect may be due to the variable abundance and composition of the prey and grazer communities.

It is interesting that the number and strength of negative associations among the three experiments varied with cladoceran biomass (Table 3). The negative effect could be due to interference competition by cladocerans, especially for *Cryptomonas*, which may be too large for

some cladocerans to ingest. It is more likely, however, that prey mortality is due to grazer ingestion. Cladocerans are known to ingest particles the size of heterotrophic protozoa and *Chroomonas*. (Carrick et al., 1991). It appears that cladocerans are the key member of the macrozooplankton community by which protozoan energy is moved up the food web to larger organisms. Given the ability of cladocerans to ingest both bacteria and heterotrophic protozoa, the strongest linkage between microbial and macroorganism food webs will occur when cladocerans are abundant.

Micrograzer feeding on protozoa and Chroomonas. Micrograzers did not measurably ingest heterotrophic protozoa in the July 94 and September 94 experiments, and in none of the experiments was there evidence that micrograzers consumed *Chroomonas* (Table 4). In the July and September 94 experiments, the majority of associations tested were nonsignificant and those that were weakly significant, were positive. In the May 95 experiment, micrograzers cleared heterotrophic protozoa at 0.3 mL/ μ g dry wt/d; the strongest association of micrograzers was with heterotrophic protozoa 12-20 μ m in length, where the clearance rate was 1.3 mL/ μ g dry wt/d ($p=0.06$). The difference between the May experiment and other two experiments was that in May, the micrograzers were dominated by rotifers. Like cladocerans, rotifers are efficient at feeding on particles the size of heterotrophic protozoa.

August 95 Experiment. Effects of macrozooplankton and nutrients on bacterial and protozoan growth. In all carboys bacterial abundance increased during the first 23 hours of incubation, then decreased substantially from 23 to 92 hours. The change in the direction of the effect at 23 hours was unequivocal and consistent across all treatments. The environments

within the carboys were obviously not constant, and therefore the incubation was divided into two parts for analysis, 0-23 hours and 23-92 hours.

During the first 23 hours bacterial growth was significantly enhanced by additional nutrients ($p=3 \times 10^{-5}$) and high macrozooplankton density ($p=2 \times 10^{-9}$); the latter factor had a greater effect (Table 5). The interaction between macrozooplankton and nutrients on bacterial growth was also significant statistically ($p=0.0004$). In the carboys with both nutrients and macrozooplankton density, elevated the mean specific growth rate was 0.546 d^{-1} , more than 7 times greater than the carboys in which both nutrients and macrozooplankton were at ambient levels. Protozoa, during the same time period, were significantly reduced by high macrozooplankton ($p=0.05$), but were unaffected by nutrients (Table 5).

The effects on bacteria and protozoa reversed after 23 hours (Table 5). Bacterial growth was reduced by additional nutrients ($p=4 \times 10^{-6}$) and high macrozooplankton density ($p=0.0001$), and the factor interaction was also significant ($p=6 \times 10^{-5}$). The greatest decline in bacterial numbers occurred in the carboys with elevated nutrients and macrozooplankton, the same carboys that had the greatest bacterial growth the first 23 hours. The least change occurred again in the carboys where both nutrients and macrozooplankton had been kept at ambient levels. Protozoa were now significantly enhanced by macrozooplankton ($p=5 \times 10^{-5}$) and nutrients remained an unimportant factor.

At first glance the outcome of this experiment appears to be a mixed bag of effects, but an interpretation is possible based on micrograzers, specifically rotifers, as the linchpin. Rotifers were more numerous in the August 95 experiment than the previous three grazing experiments, and they constituted a greater proportion of the micrograzer group (68%). Most importantly, at

the end of the experiment, rotifer abundance was inversely related to macrozooplankton abundance (Pearson correlation coefficient = -0.47, $p=0.047$). Rotifers could be reduced by interference competition from cladocerans and predation by cyclopoid copepods, both groups abundant during this experiment (Figure 3).

From 0-23 hours bacterial growth was greatest in the carboys with high macrozooplankton due to nutrient regeneration, and the effect of macrozooplankton interfering with or feeding on heterotrophic protozoa and, likely, rotifers. Rotifers were not enumerated at 23 hours, so it is unknown how the population had responded to the experimental manipulations at that time, but the downward trend noted at the end of the experiment had likely already begun. The measurable negative effect of macrozooplankton on heterotrophic protozoa may have been sufficient alone to allow the bacterioplankton population to increase. Bacterial growth increased somewhat in the carboys with a low level of macrozooplankton, probably because of reduced grazing pressure from fewer cladocerans, which were numerous in the lake in August 95.

From 23 to 92 hours heterotrophic protozoa increased dramatically, especially in the carboys with high macrozooplankton densities, presumably because the rotifers were nearly gone. Without the grazing pressure of rotifers, heterotrophic protozoa rebounded, and then in turn grazed the bacteria population to reduced levels. Bacteria were substantially reduced also in the carboys with low densities of macrozooplankton, perhaps due to unhindered bacterivory by rotifers which had their highest levels in these carboys.

The explanation offered here for the patterns in grazer and prey populations after manipulating macrozooplankton is plausible, given the observed associations among the various populations. Table 6 summarizes the associations for the two incubation periods. The

importance of heterotrophic protozoa in controlling the bacteria population is suggested by the opposite directions of their growth, consistent between the two periods. When heterotrophic protozoa go up, bacteria go down and vice versa. The abundance of heterotrophic protozoa, in turn, is affected by the abundance of their larger predators. Why protozoa decreased initially is unclear, but by the end of the experiment, a clear trophic cascade emerged, the effects of the manipulation cascading down from large to small organisms. Increasing macrozooplankton caused smaller rotifers to decrease which caused smaller heterotrophic protozoa to increase which caused bacteria to decrease. Fish were not manipulated in these experiments, but it is conceivable that altering the abundance of piscivorous fish, which would affect the zooplanktivorous fish community and consequently macrozooplankton, would ultimately alter the dynamics of bacterioplankton. Whether this would have any measurable effect on the ecological roles of bacterioplankton in Lake Champlain is unknown. Cascading trophic interactions did not occur consistently in the first three grazing experiments (Table 7) and thus the findings of the August 95 experiment cannot be generalized to the entire lake for all seasons.

Summary of Findings

1. Bacterioplankton abundance in Lake Champlain during the ice-free months was in the range of $1-2 \times 10^9$ cells/L and bacterial productivity was on the order of 10^8 to 10^9 cells/L/d. The abundance and productivity data are within normal limits, suggesting that bacterioplankton dynamics in Lake Champlain are likely typical for temperate dimictic lakes.

2. Total grazer biomass ranged from 100.3 to 1267.4 μg dry wt/L with macrozooplankton contributing the greatest amount of biomass. The macrozooplankton were dominated by either cladocerans or cyclopoid copepods. The composition of the grazer community differed among grazing experiments.
3. The cryptomonads *Chroomonas* and *Cryptomonas* are mixotrophic, motile algae that were common during the experiments and at times were the dominant nano- or micrograzer, respectively. The role of cryptomonads in the microbial food web of Lake Champlain needs further study.
4. Bacterivory could be measured once for each grazer size category at different times of the year. Bacterivory by macrozooplankton and micrograzers was measurable when these groups were dominated by taxa known to be bacterivorous, cladocerans and rotifers, respectively.
5. Clearance rates of macro, micro and nano grazers on bacteria were 1.1, 4.9, and 0.2 mL/ μg dry wt/d. Carbon fluxes from bacteria to grazers were in the range of 0.4 to 6.6 μg C/L/d.
6. Cladocerans when they are present provide a one step trophic link between bacteria and zooplanktivorous fish.
7. Enhancement of bacterial growth by macrozooplankton and micrograzers occurred when these groups were dominated by taxa that are nonbacterivorous. Nonbacterivorous taxa can promote bacterial growth through two mechanisms: nutrient regeneration and predation on smaller bacterivores release bacteria from grazing pressure.

8. Macrozooplankton clearance rates on heterotrophic protozoa ranged from 0.02 to 4.2 mL/ μ g dry wt/d and on cryptomonads from 0.02 to 0.1 mL/ μ g dry wt/d.
9. It appears that cladocerans were the zooplankter responsible for heterotrophic protozoa predation. Given the ability of cladocerans to ingest both bacteria and heterotrophic protozoa, the strongest linkage between microbial and macroorganism food webs will occur when cladocerans are abundant.
10. Measurable clearance rates by macrozooplankton on cryptomonads were obtained when cyclopoid copepods were abundant.
11. Micrograzers did not measurably ingest heterotrophic protozoa, except when rotifers were abundant. Clearance by the rotifer-dominated micrograzers on heterotrophic protozoa was 0.3 mL/ μ g dry wt/d.
12. The August 95 factorial experiment demonstrated a complex trophic cascade in which, at first, macrozooplankton enhanced bacterial growth by suppressing heterotrophic protozoa. Then later, after macrozooplankton reduced rotifers, heterotrophic protozoa rebounded after release from rotifer predation and bacteria declined quickly. In this experiment macrozooplankton predation on rotifers appeared to govern, in opposite directions, the growth of heterotrophic protozoa and bacteria. The experiment demonstrates how it is conceivable that manipulating one group of large organisms, like piscivorous fish, can have consequences for the smallest organisms, bacteria.

Citations

- Azam F, Fenchel T, Field JE, Gray JS, Meyer-Riehl LA, Thingstad F. 1983. The ecological role of water column microbes in the sea. *Mar Ecol Prog Ser* 10:257-263.
- Bott TL, Kaplan LA. 1985. Bacterial biomass, metabolic state and activity in stream sediments: Relation to environmental variables and multiple assay comparisons. *Appl Environ Microbiol* 50:508-522.
- Button DK, Robertson BR. 1993. Use of high-resolution flow cytometry to determine the activity and distribution of aquatic bacteria. In: Kempf PF, Sherr BF, Sherr EB, Cole JJ (eds): *Handbook of Methods in Aquatic Microbial Ecology*. Lewis Publishers: Boca Raton, pp 163-173.
- Carrick HJ, Fahnenstiel GL, Stoermer EF, Wetzel RG. 1991. The importance of zooplankton-protozoan trophic couplings in Lake Michigan. *Limnol Oceanogr* 36:1335-1345.
- Lehman JT, Sandgren CD. 1985. Species-specific rates of growth and grazing loss among freshwater algae. *Limnol Oceanogr* 30:34-46.
- Levine SN, Shambaugh AD, Pomeroy SE, Braner M. 1997. Phosphorus, nitrogen, and silica as controls on phytoplankton biomass and species composition in Lake Champlain (USA-Canada). *J Great Lakes Res* 23:133-150.
- Luria SE. The bacterial protoplasm: composition and organization. 1960. In: Gunsalus IC, Stanier RY (eds.). *The Bacteria*, Vol. 1. Academic Press: New York, pp 1-34.
- McCauley E. 1984. The estimation of the abundance and biomass of zooplankton in samples. In: Downing D, Rigler F (eds): *A Manual of Methods for the Assessment of Secondary Productivity in Freshwaters*. Blackwell: Oxford, pp 228-265.

- Pomeroy LR. 1974. The ocean's food web, a changing paradigm. *BioScience* 24:499-504.
- Riemann B. 1985. Potential importance of fish predation and zooplankton grazing on natural populations of freshwater bacteria. *Appl Environ Microbiol* 50:187-193.
- Ruttner-Kolisko A. 1977. Suggestions for biomass calculation of plankton rotifers. *Arch Hydrobiol Beih Ergebn Limnol* 8:71-76.

Table 1. Bacterial Numerical Abundance and Productivity in Lake Champlain During the Grazing Experiments

	July 94 Experiment	September 94 Experiment	May 95 Experiment	August 95 Experiment
Abundance				
$\bar{X} \pm SD$ (cells/L)	$2.06 \pm 0.23 \times 10^9$	$1.35 \pm 0.07 \times 10^9$	$1.14 \pm 0.05 \times 10^9$	$2.47 \pm 0.09 \times 10^9$
n	6	6	6	9
Productivity				
Specific growth $\pm SD$ (d ⁻¹)	0.57 ± 0.07	0.27 ± 0.13	0.15 ± 0.04	Unable to estimate
n	6	4	4	
P	0.001	0.16	0.07	
Cells/L/d [†]	11.71×10^8	3.7×10^8	1.70×10^8	
Grazers manipulated [‡]	Micro	Nano	Nano	

[†] Calculation assumes bacterial population is in equilibrium

[‡] The smallest size class of grazers manipulated (macro, micro, or nano) that yielded a negative association between grazer density and bacterial specific growth rate.

Table 2. Macro-, micro-, and nanograzer clearance rates on bacterioplankton in Lake Champlain. A positive sign indicates a positive association between grazer density and bacterial specific growth rate. The p and r^2 values of an association are given as superscripts and subscripts, respectively. NS indicates an $r^2 < 0.20$. Carbon fluxes from bacteria to grazers are reported in the text.

	Clearance Rate (mL/ μ g dry wt/day)		
	July 94 Experiment	September 94 Experiment	May 95 Experiment
Macrozooplankton	1.1 ^{0.13} _{0.59}	+ ^{0.19} _{0.38}	+ ^{0.06} _{0.62}
Micrograzers	NS	+ ^{0.05} _{0.66}	0.2 ^{0.14} _{0.45}
Nanograzers	NS	4.9 ^{0.35} _{0.40}	NS

Table 3. Macrozooplankton clearance and carbon flux rates on heterotrophic protozoa and cryptomonads. A positive sign indicates a positive association between macrozooplankton density and prey specific growth rate. The p and r^2 values of an association are given as superscripts and subscripts, respectively. NS indicates an $r^2 < 0.20$

Protozoan Prey	July 94 Experiment		September 94 Experiment		May 95 Experiment	
	Clearance Rate (mL/ μ g dry wt/day)	Carbon Flux (μ g C/L/day)	Clearance Rate (mL/ μ g dry wt/day)	Carbon Flux (μ g C/L/day)	Clearance Rate (mL/ μ g dry wt/day)	Carbon Flux (μ g C/L/day)
Heterotrophs						
	$1.8^{0.36}_{0.21}$	0.5	$0.05^{0.03}_{0.75}$	0.1	NS	—
	$4.2^{0.27}_{0.29}$	5.6	NS	—	NS	—
	NS	—	$0.1^{0.08}_{0.58}$	0.5	$+^{0.36}_{0.21}$	—
All Heterotrophs	$3.5^{0.27}_{0.29}$	7.4	$0.02^{0.21}_{0.36}$	0.9	NS	—
Cryptomonads						
	NS	—	$0.1^{0.002}_{0.94}$	20.8	NS	—
	NS	—	$0.02^{0.002}_{0.93}$	1.9	NS	—
	NS	—	$0.03^{0.001}_{0.94}$	9.1	$0.08^{0.17}_{0.41}$	3.6
All Heterotrophs and Cryptomonads	$3.2^{0.29}_{0.27}$	9.1	$0.03^{0.04}_{0.68}$	10.6	NS	—

Table 4. Micrograzer clearance rates on heterotrophic protozoa and *Chroomonas*. A positive sign indicates a positive association between micrograzer density and prey specific growth rate. The p and r^2 values of an association are given as superscripts and subscripts, respectively. NS indicates an $r^2 < 0.20$. Carbon fluxes from prey to micrograzers are reported in the text.

Protozoan Prey	Clearance Rate (mL/ μ g dry wt/day)		
	July 94 Experiment	September 94 Experiment	May 95 Experiment
Heterotrophs			
<5 μ m	NS	+ ^{0.23} _{0.33}	0.7 ^{0.19} _{0.38}
5-12 μ m	NS	NS	NS
12-20 μ m	+ ^{0.12} _{0.50}	+ ^{0.10} _{0.54}	1.3 ^{0.06} _{0.63}
All Heterotrophs	NS	NS	0.3 ^{0.35} _{0.22}
Cryptomonads			
Chroomonas	NS	NS	NS
All Heterotrophs and Chroomonas	NS	NS	0.2 ^{0.34} _{0.23}

Table 5. Effects of macrozooplankton abundance and nutrients on bacterial and heterotrophic protozoan specific growth rate (d^{-1}), August 95 experiment. The incubation was divided into two time periods (see text). Mean specific growth rates reported for a statistically significant factor are indicated in bold text.

Factor and Level	N	0-23 Hours				23-92 Hours			
		Bacteria		Protozoa		Bacteria		Protozoa	
		$\bar{X} \mu(d^{-1})$	P	$\bar{X} \mu(d^{-1})$	P	$\bar{X} \mu(d^{-1})$	P	$\bar{X} \mu(d^{-1})$	P
Macrozooplankton									
Ambient	6	0.091	2×10^{-9}	0.278	0.05	-0.169	4×10^{-6}	0.235	5×10^{-5}
High	6	0.429		-0.380		-0.398		1.193	
Low	6	0.152		0.121		-0.341		0.730	
Nutrients									
Ambient	9	0.171	3×10^{-5}	0.085	NS	-0.244	0.0001	0.733	NS
High	9	0.276		-0.072		-0.362		0.705	
Interaction									
Macro									
Ambient	3	0.107	.0004	0.488	NS	-0.333	6×10^{-5}	0.210	NS
Ambient	3	0.075		0.069		-0.005		0.260	
High	3	0.546		-0.747		-0.405		1.267	
High	3	0.311		-0.012		-0.390		1.118	
Low	3	0.176		0.044		-0.347		0.638	
Low	3	0.128		0.197		-0.336		0.821	

Table 6. Effect of increasing macrozooplankton on the abundance of micro- and nanograzers and bacteria, August 95 experiment. For macrozooplankton, the arrow indicates the direction of the manipulation. For the other groups, an arrow indicates the direction of an effect and that it was significant statistically (p <0.05). 0 = no effect. ?=effect unknown.

Incubation Period	Macro-zooplankton	Rotifers	Nauplii	Cryptomonas	Heterotrophic Protozoa	Bacteria
0-23 hr	↑	?	?	↓	↓	↑
23-92 hr	↑	↓	0	↑	↑	↓

Table 7. Summary of interactions between grazers and their potential prey during the three grazing experiments (Tables 2, 3, and 4). No interaction (0) is reported when the proportion of variance in prey growth explained by grazer density is < 20%. Because heterotrophic protozoa are a subset of nanograzers their interaction cannot be determined.

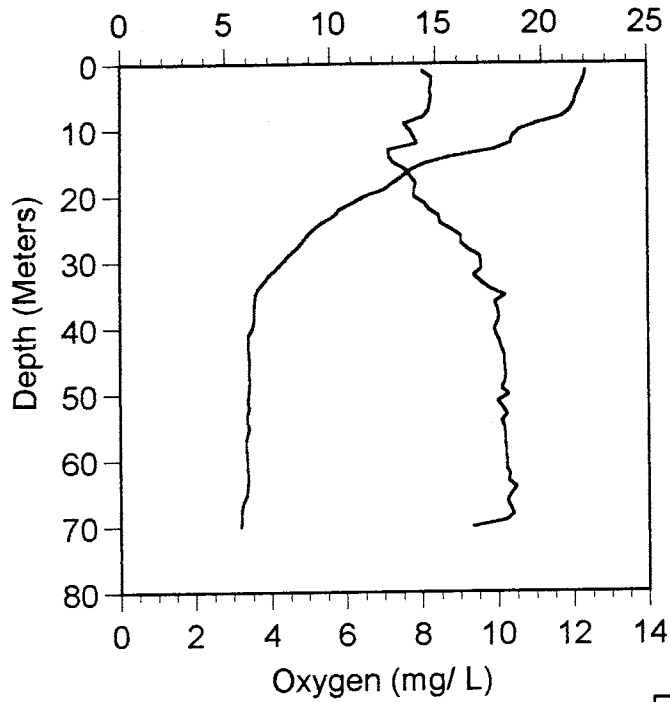
	Prey	Grazer		
		Macro	Micro	Nano
July 25, 1994	Heterotrophic protozoa	-	0	
	Bacteria	-	0	0
September 27, 1994	Heterotrophic protozoa	-	0	
	Bacteria	+	+	-
May 8, 1995	Heterotrophic protozoa	0	-	
	Bacteria	+	-	0



5-34

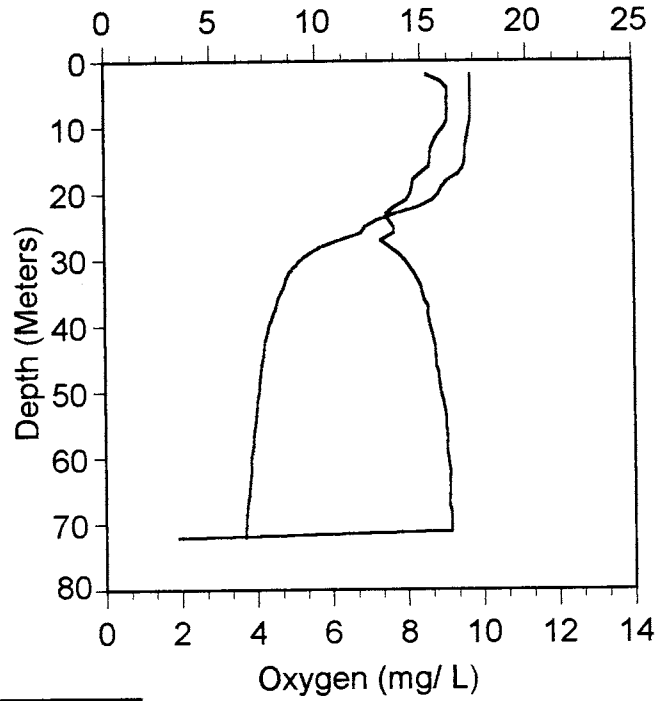
July 94 Experiment

Temperature °C



September 94 Experiment

Temperature °C

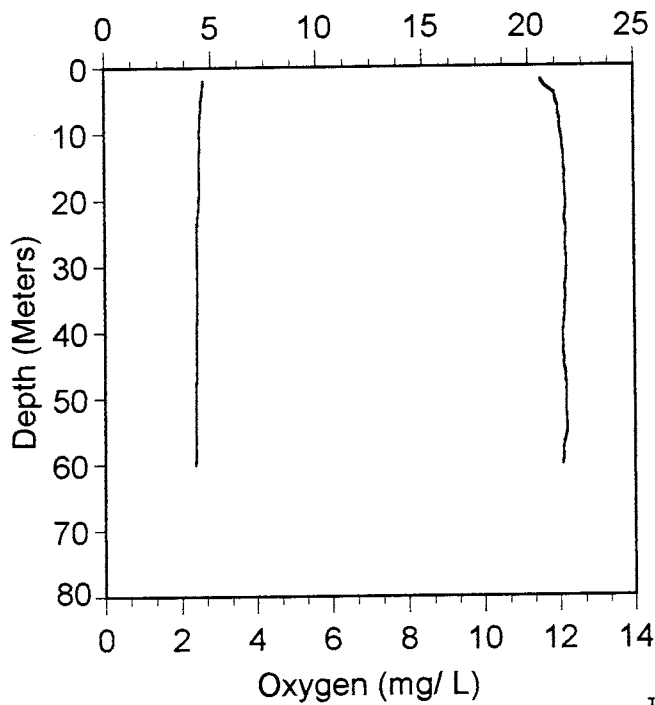


— Oxygen

— Temp

May 95 Experiment

Temperature °C



August 95 Experiment

Temperature °C

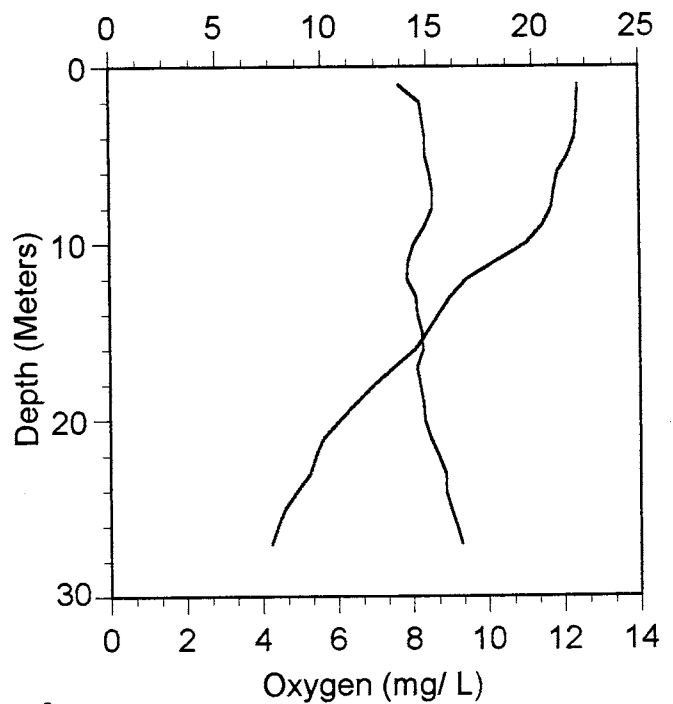


Figure 2

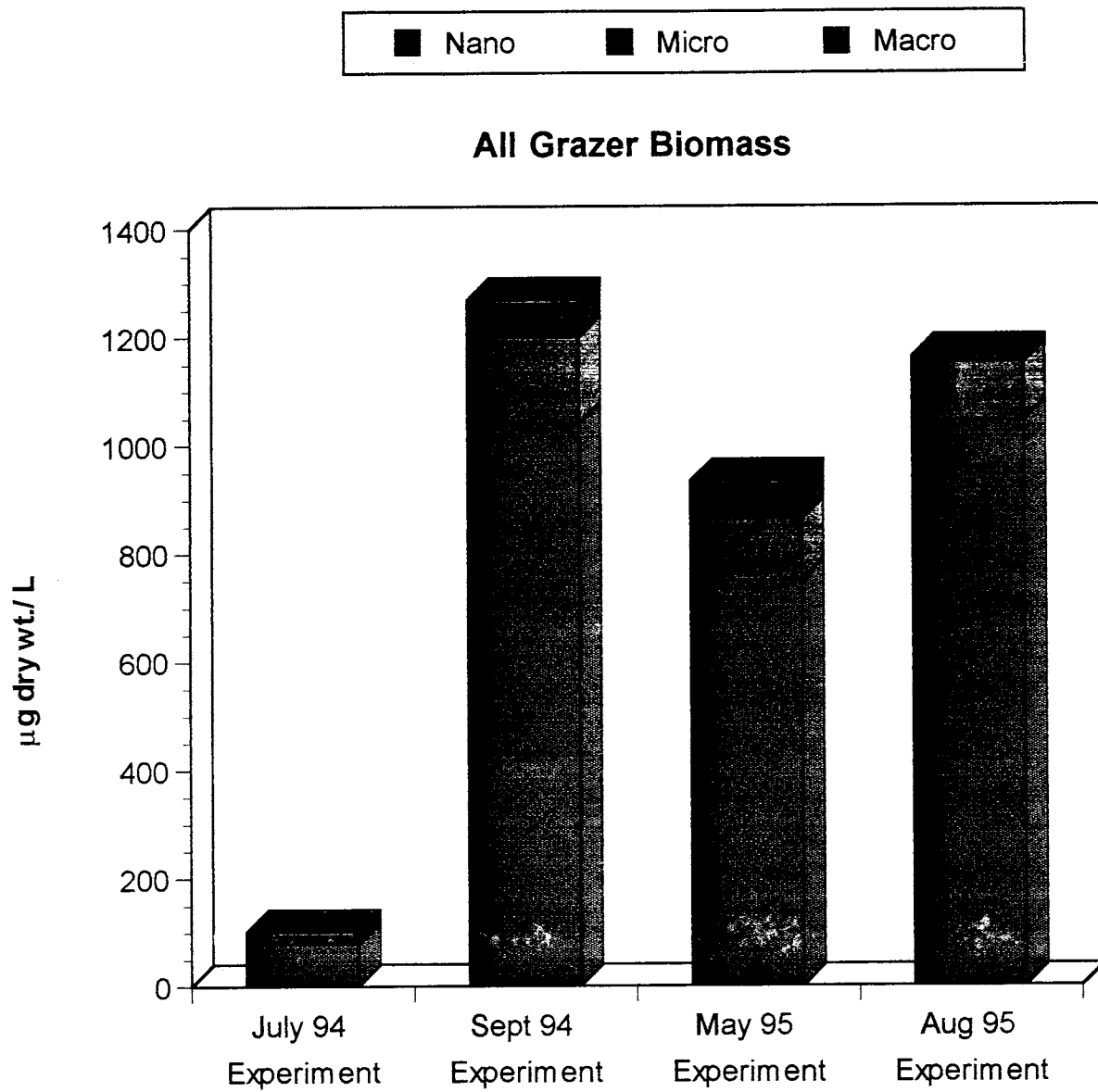
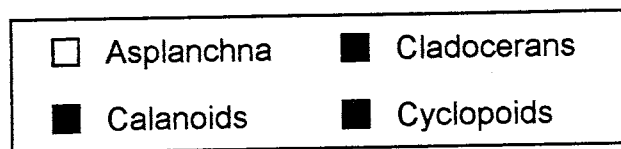


Figure 3



Macrozooplankton Biomass

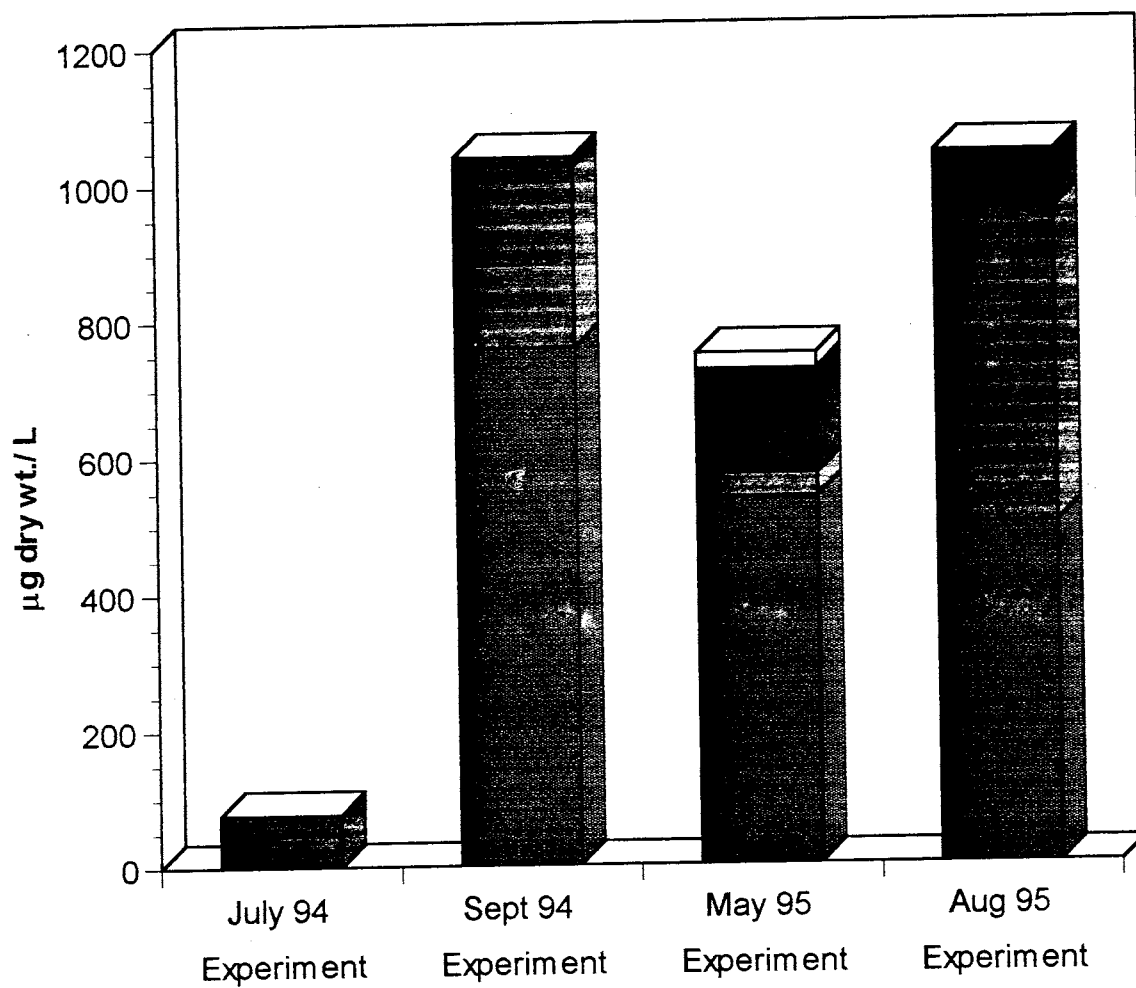


Figure 4

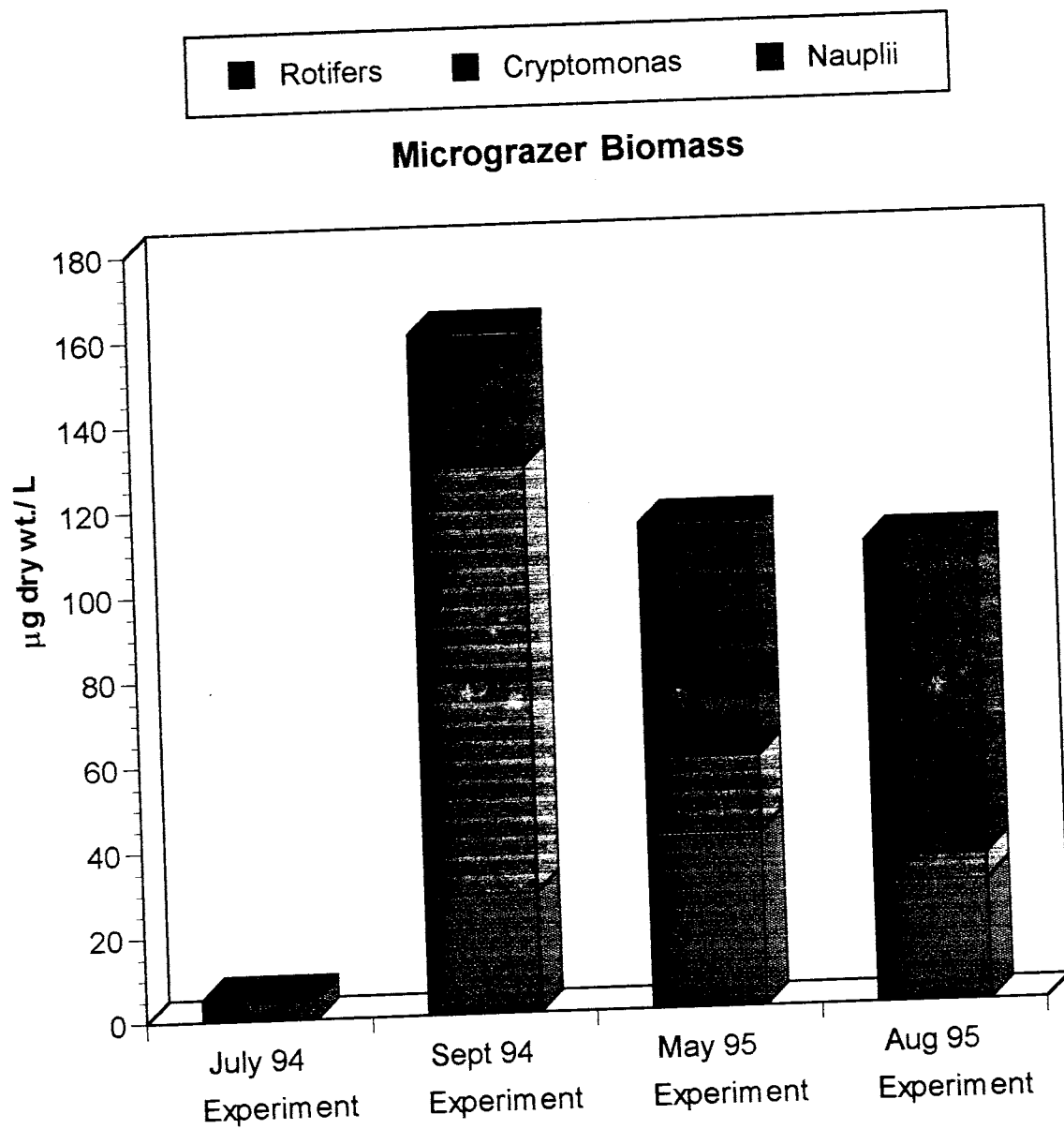


Figure 5

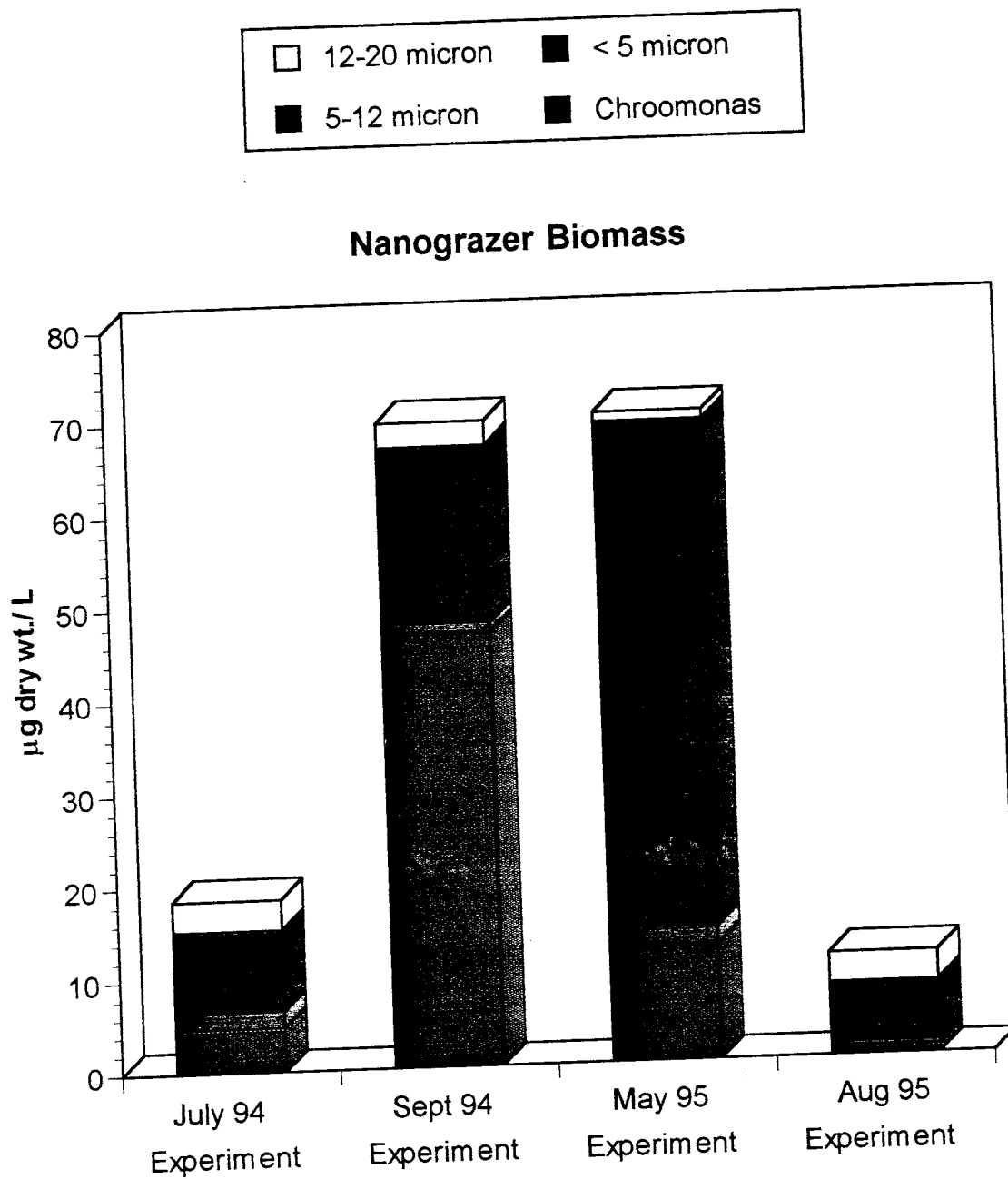


Figure 6

Section VI: Within-Season Foodweb Model

by Moshe Braner

INTRODUCTION

Purpose and Scope

The populations comprising the food web within a lake change dramatically over the course of a season. When comparing a variety of lakes, some broad correlations can be seen between annual (or summer) mean population levels and various factors such as nutrient loading or predation pressure. But the use of such means is a crude tool for understanding the dynamics operating within a season, or the reasons for a specific lake deviating from the broad trend. Within our effort to understand Lake Champlain in particular, a more refined approach is necessary.

This model is a preliminary attempt to organize thoughts on the interactions of some of the components of the lake's community as they shape the dynamics within a season. The scope of this model does not allow useful extrapolation to multi-year time scales. Winter turnover makes nutrients available for an early spring algal bloom. The dynamics within the next several months are, mathematically speaking, a particular transient trajectory of a system that would eventually settle on a much different equilibrium or sustained oscillations -- if it weren't for the effect of the next winter. The transient dynamics reflect both bottom-up effects of the released nutrients, and top-down effects of predation. Within that framework, populations respond in the time scales determined by their biological characteristics. The complete system is too complex to grasp intuitively, and a formal model is needed.

To be useful, though, the model needs to be deliberately kept simple enough for analysis. The number of system components explicitly modeled must be kept small. Some

other features of the system can be included in a simplified form as parameters affecting the dynamics of the components modeled explicitly. And many details need to be ignored altogether in the model. The goal is to capture the important details and ignore the details that are unlikely to greatly influence the conclusions.

Even with only a small number of system components in the model, the number of ways in which they can interact is fairly large. This translates into a multitude of parameters in the model for which we need numerical values in order to actually run the model. Most of these parameter values are not known for Lake Champlain. Many have been studied in the literature, but the studies produced a wide range of values, and it is not clear which values are appropriate for Lake Champlain. The exact values of some parameters have a relatively weak effect on the model behavior. On the other hand, the model behavior is quite sensitive to the values of some other parameters.

As a result of constructing and using a model of the type envisioned here, one cannot yet make precise predictions of the future nor fine-tune management actions. Rather, the model findings can point to possible lake behaviors, and to the parameters that most strongly affect that behavior. This is useful in generating qualitative predictions, and for the purpose of identifying important components for further research.

Model System Components

The following components of the food web are modeled as explicit compartments in the model:

- Phytoplankton carbon and limiting nutrient (P) contents, as separate quantities. Keeping track of these as two values allows for correct mass balance in this and higher trophic levels.
- Zooplankton carbon and nutrient (P) contents, as separate quantities. Here “zooplankton” refers to the herbivorous fraction of the zooplankton.

- Carnivore carbon and nutrient (P) contents, as separate quantities. This compartment includes invertebrate predators, in the sense that the model assumes quick growth of the carnivore biomass in the presence of adequate prey. Some life stages of some zooplanktivorous fish may be includable in this compartment.

The following components of a real food web are included in the model only indirectly, in the sense that some of their effects are taken into account, but there are no model compartments dedicated for their representation:

- Fish. The assumption is that fish are manipulated (either stocked directly, or are under heavy predation pressure by stocked fish), and thus do not respond to the available prey in the same way as the other carnivores. The model accepts fish predation pressure as an external driving function. It is assumed that the fish eat both the herbivorous and the carnivorous zooplankton.
- Benthic organisms. The model is mostly concerned with the pelagic, epilimnetic portion of the system. The activity of benthic organisms appears in the model only in the sense that the rate of mineralization of detritus that falls to the bottom is, in the real world, dependent on the benthic community.
- Bacteria and protozoa. At this stage we don't have much data that would allow for the explicit modeling of the activity of these organisms. Moreover, the time scale of their activity is faster than that of the rest of the system. In the current model, it is assumed that nutrients excreted by the modeled components are quickly made available for uptake by phytoplankton, an implicit product of bacterial activity. The portion of the reduced carbon flow from primary production to higher trophic levels via the "microbial loop" is ignored in the model for now. This may need to be modified.
- Fate of excreted carbon. In the model it is not tracked further, once excreted. It is assumed, in other words, that the energy in this carbon does not get recycled back into the modeled components of the system. Some of this energy may be transferred to higher levels via the "microbial loop", but, as mentioned above, the current version of the model

only considers the recycling of the limiting nutrient. Our preliminary experimental results for Lake Champlain show that the bacterial productivity is a nontrivial portion of primary productivity. If bacteria turn out to be an important food source for zooplankton, this aspect of the model will need to be revised.

Our model is generally patterned after that used by Ross et al (1993). That model was for a Scottish fjord system, heavily influenced by the open sea. Our model has been reshaped to better describe an inland lake, and simplified. The main differences of our model from the model of Ross et al:

- They assumed the limiting nutrient is nitrogen. In Lake Champlain the limiting nutrient is presumably phosphorus (P).
- Light limitation is not considered in our model. Although algae in the lower parts of the epilimnion may be light limited during the spring bloom due to shading by the algae above them, the algae productivity as a whole is high at that time. The light limitation of the form used by Ross et al is not appropriate here in any case, it is not a self-shading model.
- They have a complex, three-layer model of the water body. Our model has no "intermediate" layer.
- They modeled the surface (epilimnetic) water layer as having a specific volume. We perform all calculations per unit volume. Quantities in sediment and bottom water layers are expressed per unit volume of surface layer. This implicitly assumes that the volume of the surface layer is constant. Although the epilimnion depth increases somewhat over the summer, from about 10 to 15 meters, this change is not large enough to necessitate representation in the model at its current level of detail.
- Unlike a fjord with its tides, the lake system is closed except for nutrient input in runoff.
- We added additional mortality (to both herbivorous and carnivorous zooplankton) due to fish.

- They tracked excreted (organic) nutrient as a separate compartment, introducing a delay before these nutrients become available to phytoplankton. We assume that those nutrients are immediately usable (treated as inorganic). This reduces the model complexity.
- They expressed a per-uptake nutrient leakage from phytoplankton. We do not, for simplicity. Given the losses to death and grazing, plus nutrient leakage per unit biomass, the inefficiencies in the nutrient uptake process itself do not seem important to the model.
- They assumed that the per-phytoplankton-biomass leakage rates for carbon and nutrient are equal. We assume that the phytoplankton are better at retaining the limiting nutrient than carbon. Often, phytoplankton excrete reduced carbon under conditions of plentiful light coupled with nutrient scarcity.
- They adjust all biological rates according to temperature. We do not. If all processes respond to temperature in the same way, the "time" variable in the model can be thought of as "biological time", which speeds up or slows down relative to calendar time in a temperature dependent way. But if different biological processes respond quite differently, then temperature should be added to the model as an explicit external driving force. It is premature to add that to our model at this point.

METHODS

Model structure

The model is a set of first-order nonlinear differential equations. Such a system of equations can produce a wide variety of behaviors, from simple equilibrium to oscillations to "chaos". Non-differential dynamics, such as time delays, can be approximated via additional system variables with slow dynamics. In our model, an example of that is the dynamics of nutrients that quickly fall (as corpses and feces) to the sediment layer, but are released only slowly.

State Variables		
Symbol	Quantity represented	typical initial value ($\mu\text{g/l}$)
Cc	carnivore carbon concentration	1
Nc	carnivore nutrient concentration	0.03
Cz	zooplankton carbon concentration	3
Nz	zooplankton nutrient concentration	0.1
Cp	phytoplankton carbon concentration	10
Np	phytoplankton nutrient concentration	0.2
Fj	nutrient concentration in "sediment"	5
Fb	nutrient concentration in hypolimnion	2
Fs	nutrient concentration in epilimnion	2

All state variables are expressed in the units of $\mu\text{g/l}$ (same as mg/m^3), of either carbon (C) or the limiting nutrient (P). In the case of the sediments and the bottom water layer, the (nutrient) concentrations represented are *not* those that would be measured in those layers. Instead, the quantity is expressed as the concentration that would be produced in the epilimnion if the material from the lower layer were to be transported to the epilimnion and uniformly mixed there. The reason for this method of accounting is to allow correct mass balance without undue complexity in the equations. The model explicitly handles biological activity in the epilimnion only, thus there is no need to refer to the true concentrations in the lower layers.

A model run must start with given initial conditions, i.e., starting values for the state variables. Any set of values can be used to see the model response. In practice, though, I have concentrated on starting with values thought to represent possible spring conditions of low population values and high nutrient concentrations.

External driving functions also need to be defined. These can be constants, or time-dependent. The model takes into account:

- FR Nutrient concentration in runoff entering the lake ($\mu\text{g/l}$). The model assumes a constant rate of water replacement in the lake. To simulate the variable rate of nutrient

addition to the lake via runoff, the effective concentration could be varied over time, to represent both the varying runoff rate and the actual variability of nutrient concentration in river water. For now, I used $20 \mu\text{g/l}$. With the assumed water replacement rate of 0.001 per day, this corresponds to a P loading rate of 20 ng/l/day , about equal to the actual rate determined by Smeltzer and Quinn (1996). (They expressed it as 133 mt P per year, into the main lake which has a volume of about 16.8 billion cubic meters.)

- TTO Volume exchange rate for turnover events (per day). This is supposed to represent unusual turnover events such as major windstorms. In the basic model runs I simply leave this value as zero at all times. The model includes, separately, a background rate of mixing across the thermocline. This rate is presumably fairly high in Lake Champlain due to the common seiches caused by normal wind patterns.
- TSR Storm runoff input (per day). Like the unusual turnover events, this function allows the simulation of unusual runoff events. Otherwise it is set to zero.
- FPR Fish predation rate (per day). I have set this to a constant (0.03 per day), but the varying feeding rate of fish over the season can be introduced into the model via this function.

Once all the external forces have been defined, the model development over time can be computed via the integration of the differential equations, described in the next section. The integration continues until a prescribed length of time (200 days) is simulated.

In model runs, it is necessary to keep track of both the state variables and some process rates. Otherwise, reasonable looking abundances can be generated by model runs with parameter values that predict widely unreasonable process rates (Scavia et al, 1988). I have arranged for the model software to record and output the levels of net primary production and aggregate grazing rate (of zooplankton on phytoplankton).

Model Equations

To proceed from one time point to the next, the simulation computes the time derivatives of the state variables, based on the current values of the state variables and

driving functions. To organize the computations better, some intermediate quantities are calculated first. Then the time derivatives of the state variables can be computed.

Intermediate Quantities	
Fraction of carnivore uptake assimilated:	$A_c = 1.0 - F_c - E_{uc};$
Fraction of (herbivorous) zooplankton uptake assimilated:	$A_z = 1.0 - F_z - E_{uz};$
Zooplankton nutrient quota:	$Q_z = N_z / C_z;$
Phytoplankton nutrient quota:	$Q_p = N_p / C_p;$
Fractional loss of zoo biomass (per day):	$M_z = D_z + E_{bz} + FPR;$
Fractional loss of carnivore biomass (per day):	$M_c = D_c + E_{bc} + FPR;$
Total fish nutrient uptake rate ($\mu\text{g P per l per day}$):	$t_{fp} = FPR * (N_z + N_c);$
Total uptake of zooplankton carbon by carnivores ($\mu\text{g C per l per day}$):	$\gamma = C_c * G_{mx} * C_z / (C_z + H_z);$
Total uptake of phytoplankton carbon by zooplankton ($\mu\text{g C per l per day}$):	$G_s = C_z * G_{mx} * C_p / (C_p + H_p);$
Production rate of corpses & feces ($\mu\text{g P per l per day}$):	$P_{ld} = F_c * Q_z * \gamma + F_z * Q_p * G_s + D_c * N_c + D_z * N_z + D_p * N_p + F_f * t_{fp};$
Total uptake of carbon by phytoplankton ($\mu\text{g C per l per day}$):	$U_c = C_p * R_{max} * ((Q_p > Q_{min}) ? (1 - Q_{min}/Q_p) : 0);$
Total uptake of nutrient by phytoplankton ($\mu\text{g P per l per day}$):	$U_f = C_p * (U_{max} / (1 + \exp(((Q_p - Q_{max})/Q_{off})))) * (F_s / (F_s + H_f));$
Total rate of nutrient excretion by carnivores ($\mu\text{g P per l per day}$):	$X_c = E_{uc} * Q_z * \gamma + E_{bc} * N_c;$
Total rate of nutrient excretion by zooplankton ($\mu\text{g P per l per day}$):	$X_z = E_{uz} * Q_p * G_s + E_{bz} * N_z;$
Total rate of nutrient excretion by phytoplankton ($\mu\text{g P per l per day}$):	$X_p = E_{np} * N_p;$
Total rate of nutrient excretion by fish ($\mu\text{g P per l per day}$):	$X_f = E_{uf} * t_{fp};$
Net flux of nutrient from bottom layer to surface layer ($\mu\text{g P per l per day}$):	$M_{fbs} = (T_{bs} + T_{TO}) * (F_b - F_s);$
Net flux of nutrient into lake from runoff ($\mu\text{g P per l per day}$):	$W_{firs} = (T_{rs} + T_{SR}) * (F_R - F_s);$

As the model run progresses (using finite time steps, automatically adjusted to maintain a given level of numerical accuracy), the values of the state variables are stored. Additionally, some other quantities may be stored for later plotting along with the state

variables. E.g., the net primary production, defined as gross phytoplankton carbon uptake minus the carbon respired or excreted ($\mu\text{g C per l per day}$): $\text{NPP} = \text{Uc} - \text{Cp} * \text{Ebp}$; and the total zooplankton uptake of phytoplankton carbon, Gs ($\mu\text{g C per l per day}$).

Time derivatives	
Carnivore biomass (carbon) rate of growth, as uptake minus losses:	$d\text{Cc}/dt = \text{Ac} * \text{gamma} - \text{Mc} * \text{Cc};$
Carnivore net nutrient uptake rate:	$d\text{Nc}/dt = \text{Ac} * \text{gamma} * \text{Qz} - \text{Mc} * \text{Nc};$
Zooplankton biomass (carbon) rate of growth, as uptake minus losses:	$d\text{Cz}/dt = \text{Az} * \text{Gs} - \text{Mz} * \text{Cz} - \text{gamma};$
Zooplankton net nutrient uptake rate:	$d\text{Nz}/dt = \text{Az} * \text{Gs} * \text{Qp} - \text{Mz} * \text{Nz} - \text{gamma} * \text{Qz};$
Phytoplankton biomass (carbon) rate of growth, as uptake minus losses:	$d\text{Cp}/dt = \text{Uc} - (\text{Dp} + \text{Ebp}) * \text{Cp} - \text{Gs};$
Phytoplankton net nutrient uptake rate:	$d\text{Np}/dt = \text{Uf} - (\text{Dp} + \text{Enp}) * \text{Np} - \text{Gs} * \text{Qp};$
Net rate of accumulation of nutrient on bottom:	$d\text{Fj}/dt = (1 - \text{alpha}) * \text{PI}d - \text{Kjr} * \text{Fj};$
Net rate of change of nutrient concentration in bottom layer:	$d\text{Fb}/dt = \text{alpha} * \text{PI}d + \text{Kjr} * \text{Fj} - \text{Mfbs};$
Net rate of change of nutrient concentration in surface layer:	$d\text{Fs}/dt = \text{Xp} + \text{Xf} + \text{Xc} + \text{Xz} - \text{Uf} + \text{Wf}rs + \text{Mfbs};$

Model Parameters

There are many parameters used in the equations above. These are assigned constant values, to be used throughout a model run. They are grouped in this section by the system compartment they are related to. Although a specific value is listed for each parameter, I have used a number of values for trial runs. The listed value is only an example. In most cases the available estimates from our experiments and from the literature cover a fairly wide range of values.

Phytoplankton parameters: We assume that, besides a certain fraction of the algal cells dying each day, the surviving cells lose some carbon (to respiration and also leakage) and, to a lesser extent, lose nutrients to leakage. They take up the limiting nutrient according to Michaelis-Menten kinetics with maximum rate and half-saturation constants. This uptake is decoupled from growth, i.e., nutrient storage ("luxury uptake") is allowed.

But, as the phytoplankton nutrient content approaches a certain maximum level, the uptake rate declines rapidly. The phytoplankton grow, i.e., take up newly fixed carbon, at a rate that depends on their nutrient quota: below a certain threshold level, no growth occurs. Above it, growth rate increases up to some maximum.

Zooplankton parameters: We assume somewhat simpler dynamics for the zooplankton than the phytoplankton. Growth is a Michaelis-Menten function of phytoplankton availability. Although nutrient content is kept in a separate state variable, loss of nutrient is assumed proportional to loss of carbon. Perhaps this should be modified.) We assume that the zooplankton release a fraction of their intake due to "messy feeding". At high densities it is assumed that the handling of the food slows the grazing down, reducing the clearance rate. The maximum zooplankton grazing rate of 2 (C_p per C_z per day) allowed by Ross et al (1993) seems high, but they are talking about a different community (brackish fjord). Note that the ratio of the grazing parameters, G_{max}/H_p , is the clearance rate (l per μg C_z per day) at low phytoplankton density. The clearance rate needs to be multiplied by the zooplankton abundance (C_z , $\mu g/l$) to arrive at the total grazing pressure as felt by the phytoplankton (a dimensionless portion per day).

Carnivore parameters: The carnivore dynamics are similar to that of the zooplankton, except that their prey is the zooplankton rather than the phytoplankton. The fraction of carnivore biomass excreted (or respired) was set at 0.75 (per day) by Ross et al (1993), which seems very high to me. Similarly their value of 15 for maximum predation rate (C_z per C_c per day).

Other parameters: To complete the modeling of nutrient cycling, we assume that: Corpses and feces, soon after they fall down to the bottom, release a fraction (α) of their nutrient contents to the bottom water layer. The rest gets remineralized more slowly. Nutrients are returned to the epilimnion through normal and episodic mixing across the thermocline. This mixing rate is fairly high, due to seiches in Lake Champlain (Tom

Manly, pers. com.). New nutrients enter the lake via runoff – water turnover time is about 3 years. Fish prey on zooplankton and a fraction of their uptake is defecated or metabolized.

Parameter	value used here	Scavia et al (1988)	Ross et al (1993)
Phytoplankton parameters:			
death rate (includes sinking rate) (per day)	$D_p = 0.05$	0.08 for diatoms, 0.005 for flagellates and blue-greens, sinking only (recomputed here based on a 10-meter epilimnion depth)	0.1
fraction of phytoplankton carbon excreted (or respired) (per day)	$E_{bp} = 0.15$		0.25
fraction of phytoplankton nutrient excreted (per day)	$E_{np} = 0.03$		
half saturation dissolved nutrient concentration ($\mu\text{g/l}$)	$H_f = 1.0$	2.5 for diatoms, 1.0 for flagellates, and 1.3 for blue-greens	
maximum phytoplankton nutrient quota (P/C)	$Q_{\max} = 0.04$		
minimum phytoplankton nutrient quota (P/C)	$Q_{\min} = 0.01$		
storage switch function transition width	$Q_{\text{off}} = Q_{\max}/10$		
maximum phytoplankton growth rate (per day)	$R_{\max} = 1.0$	1.5 for diatoms, 1.1 for flagellates, and 0.4 for blue-greens	1.2
maximum phytoplankton nutrient uptake rate (P per C per day)	$U_{\max} = 0.1$		1.2 for Nitrogen uptake
Zooplankton parameters:			
zooplankton death rate (per day)	$D_z = 0.02$		0.05
fraction of zooplankton uptake defecated (dimensionless)	$F_z = 0.1$		0.36
fraction of zooplankton uptake excreted (dimensionless)	$E_{uz} = 0.1$	overall assimilation efficiency 0.5	0.15

Parameter (continued)	value used here	Scavia et al (1988)	Ross et al (1993)
fraction of zooplankton biomass excreted (or respired) (per day)	Ebz = 0.03	up to 0.25 for Daphnia and 0.11 for Diaptomus	0.05
maximum zooplankton grazing rate (Cp per Cz per day)	Gmax = 0.5	0.6 for Daphnia and 0.25 for Diaptomus	2
half saturation phytoplankton carbon concentration ($\mu\text{g/l}$)	Hp = 100.0	80 for Daphnia and 20 for Diaptomus	150
Carnivore parameters:			
carnivore death rate (per day)	Dc = 0.03		0.05
fraction of carnivore uptake defecated (dimensionless)	Fc = 0.3		0.5
fraction of carnivore biomass excreted (or respired) (per day)	Ebc = 0.05		0.75
fraction of carnivore uptake excreted (dimensionless)	Euc = 0.2		0.2
maximum predation rate (Cz per Cc per day)	Gamx = 0.5		15
half saturation zooplankton carbon concentration ($\mu\text{g/l}$)	Hc = 50.0		500
Other parameters:			
labile fraction of corpses & feces (dimensionless)	alpha = 0.2	0.5	0.2
remineralization rate of nutrient in sediment (per day)	Kjr = 0.02	0.01	0.01 (for Nitrogen)
fraction of fish uptake defecated (dimensionless)	Ff = 0.2		
fraction of fish uptake excreted (dimensionless)	Euf = 0.2		
normal water mixing between layers (per day)	Tbs = 0.02		
normal runoff input (and output) (per day)	Trs = 0.001		

Model Implementation

The model was written as a computer program in the "C" language. The main module is kept concise by linkage to additional modules that hold standard technical

details such as dynamic memory allocation, the actual numerical integration, output of numerical data in matrix form, and so on. The author has developed those other modules. The integration module is based on the Runge Kutta fourth order integration algorithm with automatic step sizing, as described in Press et al (1986).

The author used the Borland "Turbo C" compiler, version 2.0 for MS-DOS, to compile the code. This is an old compiler that, on modern PCs, compiles a small program, such as this one, virtually instantly. Any other standard C-language compiler may be used, the source code does not use any nonstandard features.

Initial conditions and parameter values are entered directly into the "C" source code. The locations that need to be edited are clearly marked in the source code by appropriate comments. Although the program could easily be modified to allow entering the values from a separate file, in this stage of research the model structure was changed often enough that direct editing of the source code was the most practical approach.

The output of a run is a tab-delimited text file holding the values of the chosen state variables (and other monitored quantities) for each time point. This data file can be looked at in any text editor or spreadsheet application. It can be transformed into a graphical form within a spreadsheet application. For graphical output during quick rounds of model runs, the author used additional homegrown utility programs. One transforms the tab-delimited data file into another text file holding simple vector-drawing commands, and another displays the plots on the screen. The tab-delimited file remains on the disk for later importation into a spreadsheet.

RESULTS - MODEL BEHAVIOR

Equilibrium

With some parameter values, the model runs approach an equilibrium over time. With other parameter values, the model exhibits periodic behavior, i.e., sustained

oscillations. When oscillations arise, they are of the classical predator-prey type, with three trophic levels involved here. I.e., a peak in phytoplankton is followed in time by a peak in zooplankton, and a peak in carnivore abundance comes last. All this is mathematically expected of such a model. In either case, with realistic initial conditions and parameter values, the equilibrium or the regular oscillations do not arise until after a time interval that represents more than one growing season. Given that the winter mixing of the lake, and other seasonal effects, perturb the system in major ways, we can expect the system to spend the bulk of each growing season in transient behavior. This transient behavior is very different from the asymptotic behavior the same system would eventually exhibit had it been free from perturbation.

Nevertheless, it is interesting to ask what endpoint the system evolves towards while it is not perturbed. To some extent we can solve the model equations analytically for the equilibrium. (The equilibrium may not be stable though, when oscillations are expected.) But the equations, being nonlinear, are not completely solvable. We can say that, in this model, the equilibrium abundance of herbivorous zooplankton solely depends on characteristics of the carnivores – unless the carnivory is so weak as to release control of the zooplankton, in which case some other factor (phytoplankton availability) will limit the zooplankton. We can also say that there is a positive correlation between equilibrium dissolved nutrient concentration, phytoplankton nutrient quota, and phytoplankton abundance. But the direction of “control” is not clear. Numerous numerical simulations can be used to further identify relationships between the parameters and equilibrium values. Scavia et al (1988) researched post-transient model behavior for Lake Michigan, and that could be done for Lake Champlain. But, as mentioned above, the system as modeled is rarely expected to reach equilibrium within a season, so this line of research may not be very useful.

Transient dynamics

Contrary to the statement in Scavia et al (1988), the transient dynamics in our model are not very sensitive to initial conditions. As long as the initial values are within reason for the conditions early in the season (fairly low abundances), changing some values by an order of magnitude makes no difference to the qualitative shape of the transient curves. The initial growth of some components may be advanced or delayed somewhat (on the order of a week), but after that period the dynamics are similar in each case. Changes made to the dynamic parameters have a far stronger effect on the shape of the curves.

With appropriate parameter values, the model runs produce the pattern typical of the lake: a spring bloom of phytoplankton, followed by an increase in zooplankton which in turn drives the phytoplankton abundance down for a "clear water phase". The degree of depression of phytoplankton abundance in this phase depends on the relative growth rates of the phytoplankton and zooplankton, and under some parameter values there is only a slight decrease and a leveling off of phytoplankton abundance following the spring bloom. After a pronounced clear water phase the zooplankton usually decrease, pinched between the decline in food availability and an increase in predation pressure by the carnivores. This produces a resurgence of phytoplankton biomass later in the season. This latter stage may arrive too late in the season to amount to much, or may develop rapidly and oscillate in additional boom-and-bust cycles, depending on the parameter values. The real-world details such as the phytoplankton species composition would undoubtedly modify the actual lake behavior, especially late in the season. But the main features of the early and middle part of the season are fairly predictable.

Net primary production in the model shows an interesting pattern: even as the abundance of the phytoplankton varies greatly over time in most runs, their aggregate productivity varies to a far lesser extent. I.e., when the phytoplankton biomass (carbon) is lower, the productivity per unit biomass is higher. During much of the season the model

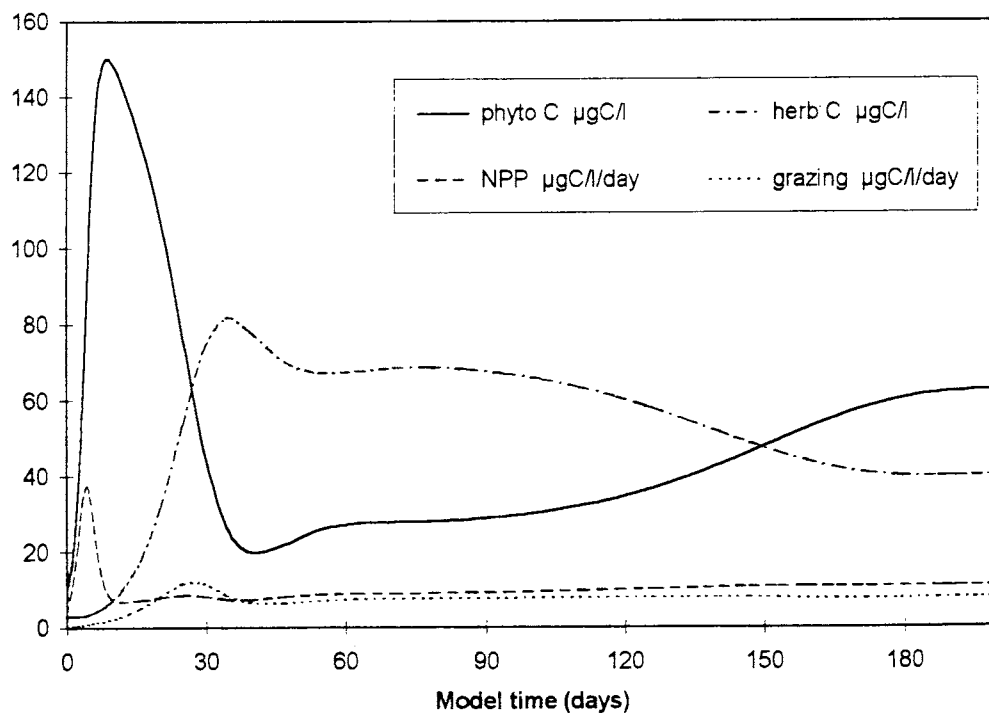
system can sustain a zooplankton biomass that is higher than the small but productive algal biomass. If this were true of the real world, the goal of clear water with lots of fish may be more attainable than one might have thought. We did estimate the zooplankton biomass as higher than the phytoplankton biomass in many instances.

Example model runs

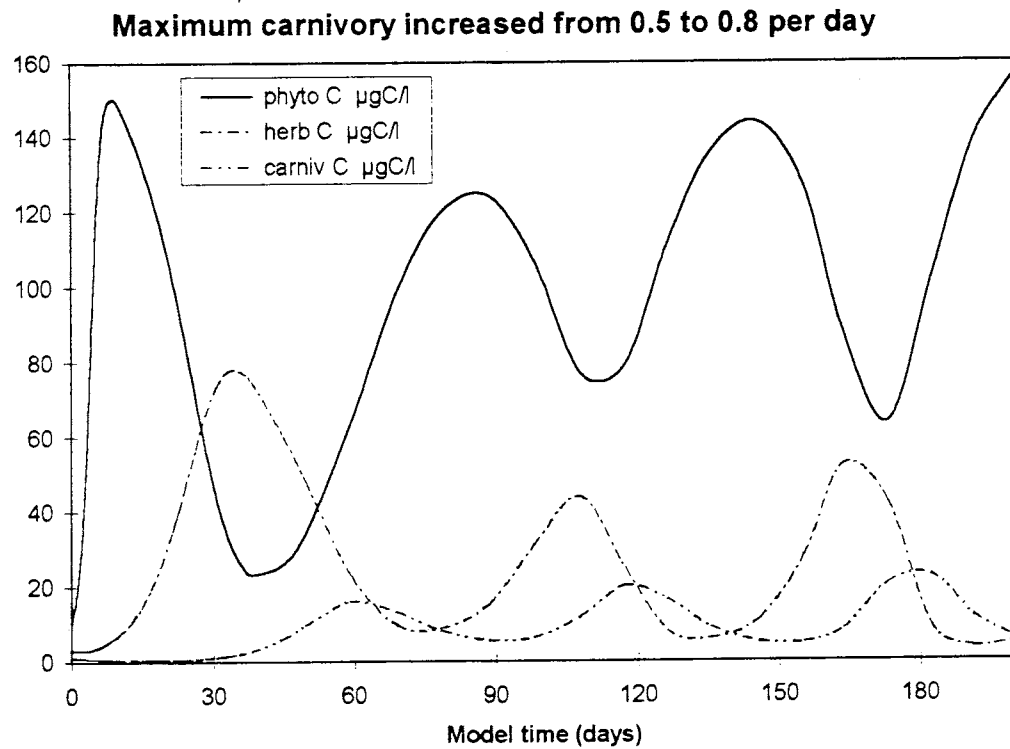
Included here are only a few example runs from the many I ran and the infinite variety that could be run. Selected state variables and other quantities are depicted to emphasize certain points, other variables could be plotted as desired.

The first example will be treated here as the “original”, with which the other ones will be compared. This one displays a simple classical spring bloom followed by a clear water phase. The parameter values used for this run are those mentioned above.

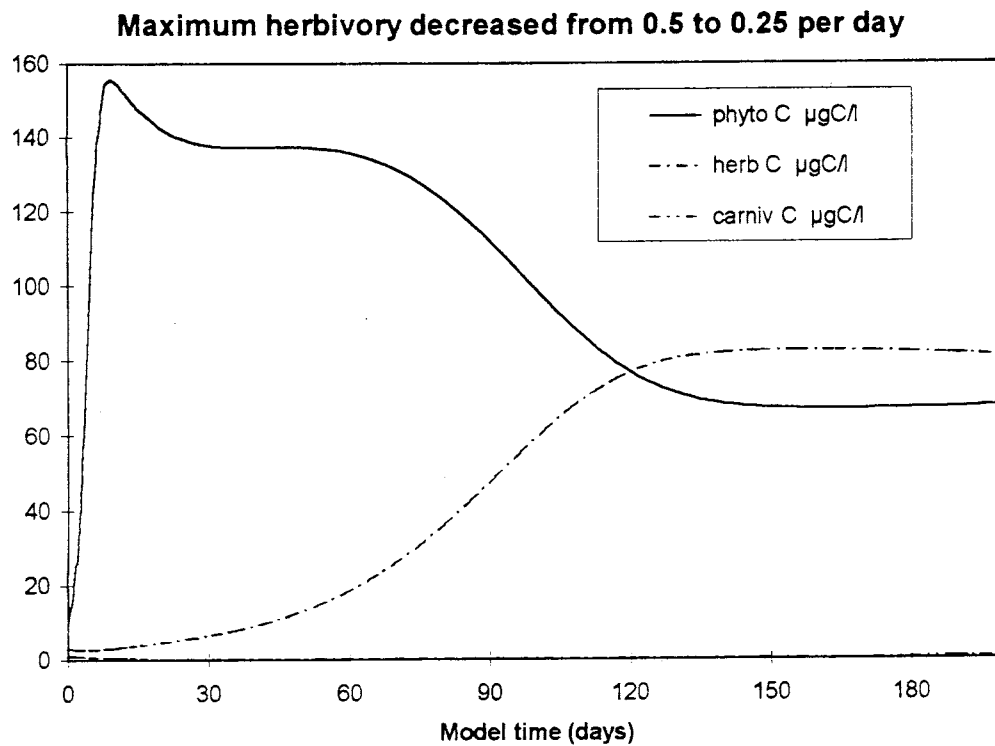
Example model run -- original initial conditions



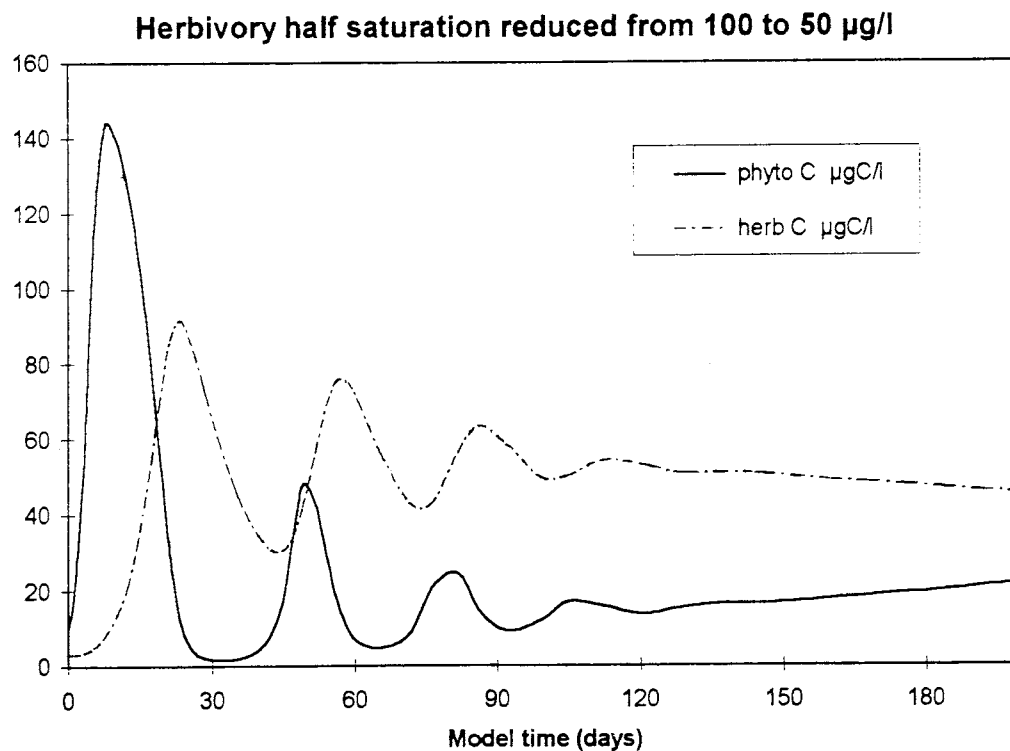
The next example run used the same initial conditions and parameter values, except that the maximum carnivory rate parameter (G_{mx}) was increased slightly, from 0.5 to 0.8 (prey C per predator C per day). The resulting destabilization is evident in the oscillation late in the season.



The third example uses the original carnivory rate but decreases the maximum herbivory rate (G_{\max}) by a half. Of note here is that there is no clear water phase, and no support for a carnivorous population, at least within the time scale of one season.



The final example is using, again, the “original” parameter values, except that this time the phytoplankton concentration needed to half-saturate the grazing function has been cut in half. This allows the zooplankton to graze the phytoplankton to a much lower level, producing a more pronounced clear water phase. Also note that, once the spring bloom is over, the herbivore biomass is generally higher than the biomass of their prey. That is a condition we have observed in Lake Champlain. It is possible thanks to high productivity of the surviving algae.



Sensitivity to parameter values

Many of the parameter values are only poorly identified at this point, or are variable over time due to changes in the biological populations or physical driving forces. One of the uses we can make of a model such as this is to determine the sensitivity of the system's behavior to each parameter. Some parameters have only a small impact on the system behavior, so that variability in their values causes little change in the quantitative, or at least the qualitative, form of the system response. Other parameters' values have stronger effects. The system behavior is strongly dependent on the exact values of a few parameters. These hint as to what might be called the controlling factors of the system, and the associated system components should be studied more closely.

One must be careful, though, when deciding how much of a change in a parameter value is appropriate when measuring the system's sensitivity to this value. To compare sensitivity across parameters, they should be perturbed to a comparable extent. But that comparability is not obvious. E.g., making a 2-fold increase or decrease in the value of each parameter may sound equitable, but some parameters, such as a nutrient quota, have a static effect, while others, such as a death rate, have a cumulative effect over time. Also, some parameters may vary a lot in nature while others don't. It is therefore difficult to rank parameters precisely on a sensitivity scale. Nevertheless, model runs show that a few parameters' values are exceptionally influential or non-influential. Following are some examples.

The concentration of dissolved, available forms of nutrients (P) in the water are often very low and difficult to measure. Therefore, the exact response of phytoplankton to these very low concentrations is not well known. From the total system perspective, though, it turns out that the half-saturation parameter (H_f) value is not at all important. Changing it by an order of magnitude has little effect on the overall system behavior. Presumably, the algae take up the nutrient as fast as it become available, keeping the

dissolved concentration small most of the time. The exact rate of uptake is not critical either, the maximum uptake rate parameter (U_{\max}) is another non-sensitive value.

Similarly, the efficiency of the nutrient uptake, in the form of nutrient leakage in proportion to the nutrient uptake, was found to be non-sensitive. This was already taken into account, I simplified the model by removing this component altogether.

The phytoplankton can take up nutrients in excess when available, and store them for later use. The extent of this storage varies and is not well known. Model behavior, though, is not sensitive to the value of the maximum nutrient quota parameter (Q_{\max}).

Nutrient cycling is important in this model, due to the fairly high rate of mixing between the surface and bottom waters. Nevertheless, the exact fraction of nutrients released immediately (α), and the mineralization rate of the rest (K_{or}), are not critical. Presumably, with enough nutrients already dissolved in the bottom waters, the mixing rate is the more critical parameter.

On the other hand, the minimum phytoplankton nutrient quota (Q_{\min}) does have a large effect. This basically expresses the nutrient material requirement needed to build an algal cell, and is better known. The maximum phytoplankton rate of growth (R_{\max}) is also a critical parameter determining system behavior.

Many of the parameters governing zooplankton and carnivore growth and death turn out to be important in determining system behavior. These include: death rates of both (D_z , D_c), fraction of biomass excreted per day (E_{bz} , E_{bc}) which can be thought of as another death rate as far as carbon accumulation is concerned, the maximum predation rate on their respective prey (G_{\max} , G_{amx}), and the half saturation constants of these two predation functions (H_p , H_z). It is these parameters that determine the time scales within which these populations respond to changes in their prey abundances, and thus whether the model behavior produces a quick or a slow transition towards equilibrium or towards oscillations.

DISCUSSION

Possible Extensions to the Model

The model described here is by no means the ultimate model of the Lake Champlain food web.

We can suggest several immediate avenues of model development.

The model can be simplified. Some processes in the current model that have shown themselves to be less important can be deleted from the model, their effects indirectly accounted for via adjustment of related parameters. E.g., the concept of maximum nutrient storage quota could be eliminated. That would remove the awkward switching function in the phytoplankton nutrient uptake expression, making the computation of equilibrium values easier. A simpler model can often capture the behavior of the system as well as the more complex one. E.g., see the later development of the model by Ross et al (1994).

The model can be made more complex, to include additional details thought important. For example, subdivide trophic levels into functional groups, as Scavia et al (1988) did. I.e., separate phytoplankton into several groups. Perhaps include limitation of diatom growth by silicon availability. Perhaps introduce phytoplankton growth rate limitation through self-shading. Separate the herbivorous zooplankton into several groups, e.g., Cladocera, Copepods, and rotifers.

Detritus in the epilimnion is ignored in the current model. Scavia et al (1988) says detrital C concentration is 5-10 times higher than algal C in Lake Michigan in the summer. Since the detrital C can be an important flow path of energy in the food web, via the microbial loop or through direct consumption by zooplankton, it needs further investigation and possible inclusion in the model. The cycling of nutrients (P) within the

epilimnion, via the detritus, is also a potentially important pathway that should be modeled.

It is necessary to identify the role of cyclopoid copepods in Lake Champlain. Are they purely carnivores, or are they partially herbivorous? With cyclopoid standing stocks often exceeding that of the other zooplankton combined, this is a key gap in our understanding of Lake Champlain. The characteristics of other carnivores, such as *Mysis*, also need further elaboration in the model.

Fish predation. I used a fixed predation pressure of 0.03 (of the zooplankton) per day. Can introduce a rate that is variable over the season. Scavia et al (1988) used a daily ration of 0.23 per day, times a fish (Alewife) carbon content of up to $2.64 \mu\text{g/l}$, resulting in a fish uptake of up to $0.61 \mu\text{g/l/day}$. With zooplankton carbon on the order of $20 \mu\text{g/l}$, the predation rate is up to about 0.03 per day, the rate I have used. In our model, though, other mortality factors, besides fish predation, are assumed to operate, and the fish predation only adds to the mortality rate. Since the rate of "other" mortality is unknown, and since the size-selective predation by fish is of no consequence to this simple model with only one predation rate on all zooplankton, the exact value of fish predation rate used is not of much importance. If and when our understanding of zooplankton mortality were to be further enhanced, there would be room for refinement of this component of the model. If the fish predation pressure has a strong seasonal cycle, that too could be introduced, providing a tighter link to the top-down models.

Lake structure. The model's caricature of the lake assumes one uniform basin. The real lake has a three-dimensional structure of multiple interconnected basins with varying depths, nutrient inputs, and physical properties such as mixing. Prediction of the real lake's behavior will have to take this structure into account.

Most importantly, in order to develop a model that has the ability to predict responses of the Lake Champlain system to management actions, more detailed data are needed.

The available estimates of critical parameters, such as grazing rates, are imprecise. Even if they were precise, they are for specific spots at specific times. The pattern over time and space is as yet unknown. Given the size and variability of Lake Champlain, getting the necessary data is a challenging and expensive undertaking. Modeling should proceed alongside further measurements, as the improvement in model usefulness will be gradual and will feed back into the planning of experimental studies.

Conclusions

We should not be surprised that the modeling results presented here are tentative and raise more questions than answers. A predictive model is a major undertaking. To quote Gaedke (1995, page 1298):

“The time scope required for model evaluation and testing depends on the particular goal since a refinement of the questions we ask demands an increase in the complexity of the models we need to answer these questions. Investigations on fully responsive and predictive models require a large and interdisciplinary team of scientists collaborating intensively for years. Apart from a well-defined scientific scope, such models require a long-term funding scheme which favours the free exchange of data and ideas.”

A comment on “control”: Scavia says the different Alewife abundances used in their model reproduced the changes in the Lake Michigan over the years, thus pointing to “top-down control”. But what controls the Alewife abundance? Until the rise and fall of the Alewife is explained, the driving forces behind the system remain unknown. In Lake Champlain, the artificial stocking of piscivorous fish may well be a controlling factor, indirectly via its effect on the zooplanktivores (mostly Smelt) (LaBar and Parrish, 1995). It is also possible for changes in the fish community to affect the species composition of the lower trophic levels, while the overall productivity is still controlled from the bottom, by the supply of nutrients and light (Armstrong 1994).

REFERENCES

- Armstrong, Robert A. (1994). Grazing limitation and nutrient limitation in marine ecosystems: Steady state solutions of an ecosystem model with multiple food chains. *Limnol. Oceanogr.* 39(3):597-608.
- Gaedke, Ursula (1995). A comparison of whole-community and ecosystem approaches to study the structure, function and regulation of pelagic food webs. *Journal of Plankton Research* 17(6):1273-1305.
- LaBar, G. W., and D. L. Parrish (1995). Bioenergetics modeling for lake trout and other top predators in Lake Champlain. Final report to New England Interstate Water Pollution Control Commission.
- Press, W.H., B.P. Flannery, S.A. Teukolsky, and W.T. Vetterling (1986). *Numerical Recipes. The art of scientific computing.* Cambridge University Press.
- Ross, A. H., W. S. C. Gurney, M. R. Heath, S. J. Hay, and E. W. Henderson (1993). A strategic simulation model of a fjord ecosystem. *Limnol. Oceanogr.* 38(1):128-153.
- Ross, A. H., W. S. C. Gurney, and M. R. Heath (1994). A comparative study of the ecosystem dynamics of four fjords. *Limnol. Oceanogr.* 39(2):318-343.
- Scavia, D., G. A. Lang, and J. F. Kitchell (1988). Dynamics of Lake Michigan plankton: a model evaluation of nutrient loading, competition and predation. *Can. J. Fish. Aquat. Sci.* 45:165-177.
- Smeltzer, Eric and Scott Quinn (1996). A phosphorus budget, model, and load reduction strategy for Lake Champlain. *Lake and Reserv. Manage.* 12(3):381-393.

SECTION VII

FINAL DISCUSSION

The project "Lower Trophic Level Interactions in Lake Champlain" was funded by the Lake Champlain Management Conference out of concern that state and federal management goals for Lake Champlain might include conflicting elements. In particular, there was concern that phosphorus control measures initiated to improve water clarity and reduce nuisance algal blooms and weed beds might ultimately affect the sports fishery by curtailing energy and nutrient flow up the foodweb. Conversely, increases in piscivorous fish populations through lamprey control and salmonid stocking could initiate a "cascade" of trophic level effects with resulting impacts on algal conditions that are not yet predictable. Under one scenario, water clarity would improve because zooplankton populations would swell as plantivorous fish succumb to the increased piscivory of the sports fish. An alternative possibility, however, is that the phytoplankton community will respond to increased grazing pressure with a switch from dominance by edible to dominance by inedible species. In this case, nuisance blooms of relatively inedible blue green algae may increase.

The zebra mussel invasion of Lake Champlain that now challenges managers had not occurred when the RFP and proposal for this project were written. Clearly zebra mussels, which feed on algae through filtering water, are likely to have an impact on the lake's foodweb as well.

Throughout the history of lake management, managers have been faced with a barrage of "ecological surprises", unexpected outcomes of their efforts due to complex food web interactions. The best strategy for avoiding these surprises is gaining familiarity with a managed lake's foodweb. The LCMC showed that it recognized this

tenet when it decided to fund three projects that addressed food web issues: the biological monitoring program that has been carried out by New York state, the top predator bioenergetics project conducted by G. LaBar (UVM) and D. Parrish (Vermont Cooperative Fish and Wildlife Research Unit, and the "bottom-up bioenergetics project" project described here. The first project provided valuable information on some of the components of the foodweb (phytoplankton, zooplankton, and zoobenthos) and how they varied spatially and over the growth season. The second contributed information on interactions between some of the piscivorous and planktivorous fish in the lake. The last project, ours, had the broadest mission of the three (and possibly of any LCMC sponsored project): to provide information on interactions between members of the planktonic foodweb other than fish, including some groups (bacteria, heterotrophic protozoa) not monitored by the NY team (and which in fact never had been assessed in the lake). Comparison of our mission with that of the monitoring program is analogous to comparing a movie with an examination of several of the movie's frames. The frames reveal major players and some scenery, but to determine the relationship between players, and the story's plot and theme, one must let the movie run over time. The monitoring program revealed the "nodes" of the lake's foodweb and their relative magnitude. Our task then was to begin the processes of identifying pathways running between "nodes" (the strings of the web, if one uses a spider web analogy) and quantifying the flow of energy and flow along these pathways. The top predator bioenergetics project had a similar task, but for just a few species. We had to deal with hundreds of phytoplankton, and dozens of zooplankton and heterotrophic protozoan, species.

The Request for Proposals which the LCMC issued in 1992 asked that the following elements be addressed in a lower trophic level study:

1. Assessment of the biomass and productivity of phytoplankton, zooplankton, bacteria and protozoa in the lake, both under ambient conditions and under varying nutrient regimes;

2. Determination of grazing rates and energy transfer between primary and secondary producers;
3. Determination of the effects of nutrient levels on species composition, size structure, and palatability of *both* phytoplankton and zooplankton communities, as these factors influence energy transfer to higher trophic levels;
4. Development of a numerical model of energy flow among zooplankton, phytoplankton and bacterial populations that might later be expanded to include fish and allow prediction of management actions.

Any one of the components of the above request (e.g., measurement of primary or secondary productivity in the lake) would easily exhaust the budget allocated for the project (\$95, 000) were measurements to be done in a comprehensive manner. Thus our response to this highly ambitious RFP, was to propose three sets of experiments that we thought would contribute substantially to understanding of the foodweb of the lake, even though they would not answer all of the questions put forth by the LCMC. These included:

1. enrichment experiments to assess nutrient limitation in the lake and differential response of phytoplankton taxa to nutrients;
2. grazing experiments that would yield estimates of energy and material flow from phytoplankton and bacteria to zooplankton, from bacteria to heterotrophic protozoa, and from heterotrophic protozoa to zooplankton (the last effects an indirect link between bacteria and protozoa), and also provide some information on primary and bacterial productivity in the lake; and
3. an experiment involving simultaneous manipulation of nutrients and zooplankton populations that would provide information on the relative importance of the two variables in controlling phytoplankton, protozoan, and

bacterial populations, and might reveal the potential for indirect effects due to top-down or bottom-up management schemes.

The foodweb model suggested by the LCMC is of course highly desirable.

Foodwebs are extraordinarily complex, so that it is difficult to predict the consequences of disturbance intuitively. A model can integrate information on the various competitive, feeding and synergistic relationships within the system and predict the direction and magnitude of "ripple effects" through the system. A predictive foodweb model clearly would be a boon for managers in the Basin. This is why the LCMC requested that we begin the model. Unfortunately, Lake Champlain is a woefully understudied lake. Almost nothing is known about the nutrient dynamics of its phytoplankton and bacteria, about light controls on phytoplankton, about the sinking rates of algae and zooplankton feces, about the rate at which nutrient-rich bottom waters are entrained into surface waters during seiches, about productivity at the various trophic levels, or about other parameters which must be quantified to run a model. Thus although we agreed to develop a model of Lake Champlain's lower foodweb, we were cautious from the start to disclaim model accuracy with regard to how closely Lake Champlain would be depicted or how useful the model would be for predictive purposes. In its current state of development, the model's principal attribute is the insights it provides about the relative importance of different processes and populations in perpetrating or dampening bottom-up or top-down ripple effects. Alterations of the values of some environmental parameters yield major changes in the model at multiple trophic levels, while other parameters can be varied by an order of magnitude or more without eliciting significant changes. Thus the model can be used to direct further research towards the more sensitive areas.

In accomplishing the objectives presented in our proposal, we were mostly successful. Some of our major accomplishments (findings) were as follows:

1. We measured primary productivity in the lake for the first time using the sensitive ^{14}C technique (which unfortunately was too expensive to permit more than two sets of measurements). The levels of productivity measured were within the range typifying a "mesotrophic" lake. If there is still a debate as to whether Main Lake is mesotrophic or oligotrophic, our data comes down on the side of the former position.
2. The nature of the photosynthesis-light relationships obtained indicated that under spring and fall conditions, phytoplankton in the Main Lake are frequently mixed to depths where little light penetrates and thus are adapted to low light conditions. Nevertheless, the mean light intensity of the mixed layer during the two experiments was less than I_k , the light intensity at which phytoplankton begin to switch from light-limited to light-saturated photosynthesis. Thus the phytoplankton in the lake probably were light limited. We did not measure primary productivity during the July experiment, but did notice that the depths of the photic zone and mixed layer were similar. Thus, even in summer, phytoplankton spend some time at water depths where light intensities are just high enough to allow growth and where photosynthesis is proportional to light intensity.
3. Our enrichment studies were the first to examine nutrient limitation of native phytoplankton communities in Lake Champlain. Algal bioassays for nutrient limitation conducted in the 1970's were based on *Selenastrum capricornutum* response to nutrient addition when grown in Lake Champlain water. We did not find evidence for persistent and strict phosphorus limitation of algal growth, as has often been suggested. Instead we found that phytoplankton responded either mildly or not at all to singular phosphorus amendments. Substantial phytoplankton growth occurred only when nitrogen and phosphorus were added in combination (and in spring, phytoplankton did not respond to any nutrient addition, indicating growth limitation by another factor, probably light). It may be that phosphorus is limiting during lake stratification but nitrogen is also very scarce, so that addition of phosphorus quickly brings on nitrogen limitation. When both nutrients were added, the double limitation hurdles were overcome, and phytobiomass

increased rapidly. It is also possible that phytoplankton growth is relatively balanced in Lake Champlain as far as nutrients are concerned. The phytoplankton community may adjust its species composition to use nitrogen and phosphorus in the proportions presented through recycling within the mixed layer (via decomposition, and zooplankton excretion), as suggested by Goldman (1980). Physiological assays of phytoplankton phosphorus status confirmed that periods of phosphorus sufficiency are as frequent as periods of phosphorus deficiency in the Main Lake.

4. We detected no symptoms of silica limitation in the lake. Even diatoms failed to respond to silica additions with increased growth. Loss of diatom dominants from lakes during eutrophication is often attributed to Si exhaustion from the water (Schleske and Stormer 1975; Tilman et al. 1981).

5. Our estimates of zooplankton grazing rates on phytoplankton indicated considerable prey selectivity on the part of the zooplankton. Not only were colonial blue green algae avoided, as the literature suggests, but many other species, including a variety of diatoms, were poorly grazed. Among the most edible algae were dinoflagellates, some common green algal species, and *Cryptomonas*. The smaller cryptomonad, *Chroomonas*, which is the most abundant species in Lake Champlain, was poorly grazed, perhaps because it is too small to be efficiently harvested. *Melosira*, the lake's spring dominant, was grazed at an intermediate rate. Thus the study showed potential for changes in the structure of the phytoplankton community as a result of top-down foodweb manipulations that alter zooplankton feeding pressure.

6. From the grazing experiments, we also learned that microzooplankton (rotifers and copepod nauplii) graze more efficiently on phytoplankton than do macrozooplankton, when the comparison is made on a weight basis. Therefore, although cladocerans and adult copepods often make up the bulk of zooplankton biomass in Lake Champlain, the smaller biomass of micrograzers present can account for much of the grazing taking place. Up until five years ago, rotifers were not included in most zooplankton studies in

Lake Champlain. Our study clearly suggests that rotifers should be given as much attention as larger zooplankton. While these animals may not be directly consumed by planktivorous fish, they may provide energy for the upper foodweb indirectly, through their consumption by invertebrate predators (e.g., cyclopoid copepods, *Mysis*, and *Leptodora*) which in turn are preyed upon by fish. Their potential impact in structuring phytoplankton communities is reason enough to monitor these animals.

7. The grazing experiments indicated that the phytoplankton community as a whole loses from about ~2-20% of its biomass per day to grazing. These losses need to be balanced by new biomass production (primary productivity) if standing stocks are to be maintained in the lake. We found that, on average, primary productivity yielded about twice as much carbon per day as was lost to grazing. The remaining carbon leaves the phytoplankton pool via other losses, such as sinking out of the mixed layer or via natural death and decomposition (Reynolds 1984). Cyr and Pace (1992) have reported grazing rates similar to ours for a large suite of northeastern U.S. lakes.

8. Analysis of bacterial populations during the grazing experiments indicated that standing stocks of these organisms are substantial, almost as great as those of phytoplankton ($28\text{-}56 \mu\text{g dwt} \cdot \text{L}^{-1}$ vs. $34\text{-}278 \mu\text{g dwt} \cdot \text{L}^{-1}$). Bacterial productivity (which could only be estimated under fertilized conditions) was temperature-sensitive, ranging from a low of $2 \mu\text{g} \cdot \text{L}^{-1} \cdot \text{day}^{-1}$ in May to a high of $17 \mu\text{g} \cdot \text{L}^{-1} \cdot \text{day}^{-1}$ in July. The May and September estimates were equivalent to 10 and 28% of the carbon fixed through photosynthesis in the lake during those experiments (primary productivity was not measured in July). Since the lake was not fertilized like the carboys, the primary productivity in carboys was probably greater and the carbon flux to bacteria somewhat less significant than the above comparisons suggest. Nevertheless, it is clear that substantial amounts of carbon are processed by bacterioplankton.

9. Grazing on bacteria was substantial. Due to fluctuating animal communities, we were able to obtain estimates of grazing on bacteria by the three grazer size classes on just one

occasion each. The rates obtained were $2.5 \mu\text{g C}\cdot\text{L}^{-1}\cdot\text{day}^{-1}$ for macrograzers (cladocerans; July 1994), $0.4 \mu\text{g C}\cdot\text{L}^{-1}\cdot\text{day}^{-1}$ for micrograzers (rotifers and nauplii; May 1995), and $6.6 \mu\text{g C}\cdot\text{L}^{-1}\cdot\text{day}^{-1}$ for nanograzers (heterotrophic protozoa; September 1994). These rates can be compared with grazing rates on phytoplankton (due to both micro and macrograzers): 29, 0.4, and $0.5 \mu\text{g C}\cdot\text{L}^{-1}\cdot\text{day}^{-1}$ in May, July and September, respectively. It appears then that the amount of carbon moving through the microbial loop from bacteria to protozoa and zooplankton is of a similar order of magnitude to that flowing up the classic food chain (from phytoplankton to zooplankton to fish).

10. Linkage of the microbial loop to the classic food chain is via zooplankton grazing on protozoan. Our estimates of C flow across this link were 0, 7.4, and $0.9 \mu\text{g C}\cdot\text{L}^{-1}\cdot\text{day}^{-1}$ during the May, July and September experiments, respectively. If these values are accurate, then it appears that zooplankton fed principally on heterotrophic protozoa rather than of phytoplankton during the clear water phase of July 1994. The C moving from bacteria to protozoa to zooplankton was probably formed during the earlier spring bloom.

11. Our nutrients versus grazers experiment was "eye-opening" in the diversity of linkages between zooplankton, phytoplankton and bacterial groups revealed. Removal of macrozooplankton (mostly carnivorous cyclopoid copepods) from carboys resulted in increased rotifer populations in the fertilized carboys and a subsequent decline in bacterial populations. The two-pronged nature of the phytoplankton-zooplankton relationship also was apparent: while in fertilized carboys, phytoplankton biomass declined with increasing grazer abundance (as one would expect given the mortality inflicted by the latter group on the former), in unfertilized carboys, the relationship between phytoplankton and zooplankton biomass at the experiment's end was a positive one. In the latter situation, nutrient recycling through zooplankton excretion apparently stimulated growth to a greater extent than it was reduced by grazing. In the fertilized systems, nutrient uptake was saturated so that the nutrient released by zooplankton had no

impact on phytoplankton dynamics. This experiment then revealed a potential for the lake to "surprise" managers with indirect responses to manipulations.

12. The nutrients versus grazers experiment also highlighted the sensitivity of both phytoplankton and bacterial populations to level of nutrient input. Two-way ANOVA indicated that there was a highly significant relationship between nutrient level and biomass, and also that nutrients were more important than grazers in determining variance in phytoplankton and bacterial densities in the experimental carboys. One should be cautious in interpreting the experiment's outcome in terms of top down versus bottom up controls, however, as the two aspects of the grazer effect (nutrient regeneration and feeding) conflicted with one another yielding a rather fuzzy overall "relationship".

13. The model indicated that the pelagic foodweb may be highly sensitive both to fish feeding, and to nutrient inputs to the lake. The values of the following model parameters were particularly important in driving the behavior of the system: minimum phytoplankton nutrient quota; maximum phytoplankton rate of growth; and zooplankton (both herbivorous and carnivorous) growth (grazing) and death (and excretion) rates. It is these parameters that determine whether the model behavior produces a quick or a slow transition towards equilibrium (following disturbance) and whether oscillations develop. Thus, changes in zooplankton species composition brought on by management actions (such as fish stocking) could have significant indirect effects. On the other hand, the values of the following parameters had relatively little effect on system behavior: the concentration of dissolved, available forms of nutrients (P) in the water, as compared with the threshold concentration for phytoplankton uptake; the exact rate and efficiency of nutrient uptake by the phytoplankton; the temporary storage of excess nutrients inside the phytoplankton; and the exact rate of nutrient release from detritus.

What are the management implications of our findings? For nutrient management, they are not serious. It appears that the lake is not as phosphorus limited as

managers have believed. Consequently, response of the lake to phosphorus cutbacks may not be as rapid as has been predicted on the basis of the assumption that phytoplankton respond to only the one limiting factor. Nevertheless, phosphorus management is the appropriate measure to be taken to reduce algal biomass in the lake. Best management practices that reduce phosphorus inputs generally result in nitrogen reductions as well, and municipal treatment of waters to remove phosphorus is much less expensive and more manageable than N removal. Even when P is not strictly limiting, reductions in its inputs may bring on phosphorus limitation. On the other hand, we suggest that managers keep "a close eye" on lake nitrogen dynamics and on N loading to the lake. Should we fail in bringing P concentrations in the lake down, the steadily increasing N inputs associated with acid rain could in time yield an increase in algal biomass. Axler et al. (1994) have reported widespread eutrophication of northern Minnesota lakes as a result of nitrate loading through acid rain. The rainfall in Minnesota contains less N than that which falls over New York and Vermont. Thus a nitrogen "threat" is clearly present.

The potential for top-down effects through fish stocking seems real, although additional work must be done to determine how much predation is necessary to drop planktivorous fish stocks and thus increase zooplankton densities. An obvious gap in Lake Champlain research has been quantification of planktivorous fish predation on zooplankton. Reviewing the information gathered to date on lakes undergoing biomanipulation (fish stocking with the intention of decreasing algal stocks), McQueen (1990) concluded that only heavy-handed piscivorous fish additions affected algae, apparently due to a dampening of effects as they move down through the various trophic levels. Thus it seems unlikely that the moderate fish stocking going on in Lake Champlain would jeopardize the eutrophication control methods being enacted on the lake. It is more likely that zebra mussels will affect phytoplankton dynamics.

We believe that it is important that the biological (and chemical) monitoring program initiated for Lake Champlain a few years ago be continued. Regardless of the

nature of the challenges the lake faces in the near future (climate change, UV radiation, zebra mussels, etc.), managers will be better prepared to meet these challenges if baseline (i.e., current normal) conditions are known. Monitoring data will provide knowledge of standing stocks, if not process rates. The monitoring program should expand, however, to include measurements of bacteria and heterotrophic protozoan densities.

In addition, we feel that more of an effort should be made to quantify processes in the lake, both grazing relationships and aspects of nutrient cycling. It would be especially prudent to examine those lake parameters that strongly influenced the behavior of our foodweb model, including phytoplankton and bacterial nutrient uptake dynamics, zooplankton nutrient excretion and grazing (continue our work, with additional attention to carnivorous zooplankton grazing on herbivores), particle sinking dynamics, and analysis of nutrient inputs through upwelling during seiches and storms. The interplay of light, phosphorus and nitrogen as limiting factors also should be examined in more detail.

Lake Champlain is appreciated for its beauty and recreational value. It should also be appreciated as a large and complex ecosystem. Understanding of this ecosystem will evolve with the various studies that take place. To understand the lake well enough to model it in a predictive manner will require many years of interdisciplinary research and consistent high level funding. It will also require a group of researchers committed to data sharing and cooperative efforts. How could such an effort be funded? With millions of dollars spent on lake-based recreation each year, one could as easily ask "How could those using the lake and caring about its health fail to fund lake research?" Mechanisms should be found to funnel tourist and recreation profits towards limnological efforts. Just a one percent profit sharing for limnology could fund a respectable effort.

References

- Axler, R.P., Rose, C., and Tikkanen, C.A. 1994. Phytoplankton nutrient deficiency as related to atmospheric nitrogen deposition in northern Minnesota acid-sensitive lakes. *Can. J. Fish. Aquat. Sci.* 51: 1281-1296.
- Cyr, H., and Pace, M.L. 1992. Grazing by zooplankton and its relationship to community structure. *Can. J. Fish. Aquat. Sci.* 49: 1455-1465.
- Goldman, J.C., 1980, Physiological processes, nutrient availability and the concept of relative growth rate in marine phytoplankton ecology, *In Primary Production in the Sea*, ed. P. Falkowski, P., p. 179-194. New York: Plenum.
- McQueen, D.J. 1990. Manipulating lake community structure: Where do we go from here? *Freshwater Biol.* 23: 613-620.
- Reynolds, C.S. 1984. *The ecology of freshwater phytoplankton*. Cambridge: Cambridge University Press.
- Tilman, D., Kilham, S.S., and Kilham, P. 1982. Phytoplankton community ecology: The role of limiting nutrients. *Ann. Rev. Ecol. Sys.* 13: 349-372.