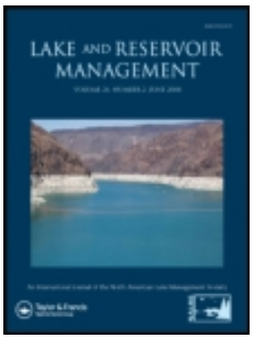

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Algal Nutrient Deficiency: Growth Bioassays versus Physiological Indicators

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ABSTRACT

The ability of nutrient enrichment bioassays versus physiological indicators to detect ecosystem changes in nutrient cycling were compared. Field data spanned four years in three lakes, two of which were subjected to fish manipulations in year two. The manipulations changed phytoplankton and zooplankton assemblages and nutrient cycling. Phosphorus and nitrogen enrichments (phosphorus enrichment bioassay (PEB), nitrogen enrichment bioassay, nitrogen plus phosphorus enrichment bioassay, specific alkaline phosphatase activity, ammonium enhancement response, and total nutrients (total phosphorus, total nitrogen) were measured before and after the manipulations. Although physiological indicators were often more sensitive, nutrient enrichments and physiological indicators both led to the same conclusions regarding changes in nutrient cycling. Total nutrients did not respond to manipulation. Physiological indicators measure the extant algal assemblage, are rapidly assayed, can be run more frequently than nutrient enrichments, and lead to the same conclusions as nutrient enrichment bioassays.

Introduction

Lake monitoring programs often quantify inputs and outputs of nutrients, particularly phosphorus, for use in predictive water quality models (Dillon and Rigler, 1974; Vollenweider, 1976). Nutrient concentrations alone, though, fail to explain the enormous range of productivity in the world's lakes (Schindler, 1978). Numerous other factors regulate algal growth, modifying cell response to nutrients (Bergquist and Carpenter, 1986; Harris, 1986; Carpenter et al. 1987). Information concerning algal nutrient limitation is central to choosing and evaluating management programs (Cooke et al. 1986). Both nutrient enrichment bioassays and physiological indicators can be used to determine the nutrient status of an algal assemblage.

Nutrient enrichment bioassays assess growth in enriched and unenriched lake water. Such bioassays are relatively easy to set up and analyze, but suffer from several problems. Samples must incubate two to four days to achieve detectable growth; during this time algal assemblage composition and characteristics can change (Vincent et al. 1984). Container effects arise from container material and size, unnatural light and mixing regimes, exclusion of grazers, and differential bacterial growth (Venrick et al. 1977; Healey, 1979; Elser et al. 1988; Elser and Kimmel, 1985; Gerhart and Likens, 1975).

Physiological indicators focus on shifts in cell metabolism that indicate internal nutrient depletion and can be rapidly measured within a few hours of sampling (Healey, 1973, 1975; Healey and Hendzel, 1980; Morris et al. 1971; Yentsch et al. 1977). These short duration assays assess the extant assemblage, and

can be measured much more often than enrichment bioassays. Physiological indicators can be nutrient-specific (e.g., alkaline phosphatase activity) or general (e.g., protein to carbohydrate ratios), allowing for flexible sampling programs. Despite the many methodological advantages of physiological indicators, species-specific responses to nutrient deficiency can make interpretation (Healey and Hendzel, 1976, 1979; Vincent, 1981), and quantitative assessment difficult (Goldman and Dennett, 1983, 1986).

Most monitoring programs are designed to detect ecosystem change. Many kinds of inadvertent perturbations and deliberate management activities could alter nutrient cycling, which may not be detectable by measuring changes in total nutrient concentrations. Among these are food web manipulations that alter the composition of zooplankton (Carpenter et al. 1987). During summer, zooplankton often satisfy algal requirements for inorganic nitrogen and phosphorus through excretion (Kitchell et al. 1979; Laird et al. 1988; Lehman, 1984). Excretion rates are strongly size-dependent (Peters, 1983) and planktivory by fishes governs zooplankton body size in lakes (Brooks and Dodson, 1965). Fish-induced changes in zooplankton body size and biomass should alter nutrient cycling and produce significant changes in nutrient deficiency indicators (Carpenter and Kitchell, 1984; Shapiro and Wright, 1984).

Whole lake fish manipulations performed to test the cascading trophic interactions hypothesis (Carpenter et al. 1987) provided a unique opportunity to examine and compare nutrient deficiency indicators. Experimentally-imposed changes in fish community structure substantially altered zooplankton body size as well as algal biomass and productivity (Carpenter et al. 1987; Carpenter and Kitchell, 1988). Previous analyses of three-to-five-day enclosure experiments and field data from one summer indicated that changes in herbivore size structure produced changes in algal nutrient status (Elser et al. 1988). Here a much larger data set was examined, spanning four years to test for long-term changes in nutrient deficiency indicators and nutrient enrichment bioassays. Randomized intervention analysis (Carpenter et al. 1989) was used to enable testing for significant changes in the indicators following the manipulations.

Methods

Manipulations

Peter, Paul, and Tuesday Lakes are small (surface area 0.8-2.4 ha), deep (max. depth 15-20 m), kettle basins located within 0.5 km of each other at the University of Notre Dame Environmental Research Center, section 36, T45N, R42W, Gogebic County, Michigan. Manipulations of the fish communities in Peter and Tuesday Lakes began in 1985 after a year of baseline study (1984) in all three lakes. In 1984, Peter and Paul Lakes contained only largemouth bass, and Tuesday Lake only winter-kill-tolerant planktivorous minnows (Carpenter et al. 1987). In May 1985, 90 percent of the adult bass from Peter Lake were exchanged for 90 percent of the minnows in Tuesday Lake, while Paul Lake remained unchanged as the reference lake. Bass survivorship was high and minnows were eliminated in Tuesday Lake, thus greatly reducing planktivory (Carpenter et al. 1987). During August 1985, zooplankton mean size increased markedly as large cladocerans, predominantly *Daphnia pulex*, became abundant. *Daphnia pulex* dominated the zooplankton through the summers of 1986 and 1987. In Peter Lake, the minnows were eliminated in less than a month by the remaining adult bass. Recruitment of the young-of-the-year bass was high and these fishes fed offshore during August and September 1985, greatly elevating planktivory (Carpenter et al. 1987). Zooplankton mean size decreased sharply between July 20 and August 10, 1985, because of intense planktivory, and relatively small-bodied herbivores dominated through the remainder of 1985 (Carpenter et al. 1987; Elser et al. 1988). However, this response was short-lived, and large-bodied daphnids prevailed in Peter Lake during the summers of 1986 and 1987 (Carpenter and Kitchell, 1988).

Limnological Analyses

All three lakes were routinely monitored from approximately mid-May to mid-September, 1984 to 1987. Epilimnetic water samples were taken from depths corresponding to 100, 50, and 25 percent of surface irradiance. Chlorophyll *a* samples from each depth were filtered onto Whatman GF/F filters, frozen, and extracted in methanol for 24 hours (Strickland and Parsons, 1968). Chlorophyll was determined by fluorometry, correcting for pheopigments. Alkaline phosphatase activity was also determined at these three depths (Pettersson, 1980). Chlorophyll-specific alkaline phosphatase activity (specific alkaline phosphatase activity = alkaline phosphatase activity/chlorophyll) is reported here as a physiological indicator of phosphorus deficiency. Chlorophyll and specific alkaline phosphatase activity results are reported as averages of the three epilimnetic depths.

Ammonium enhancement response was determined as a physiological indicator of nitrogen deficiency (Yentsch et al. 1977; Elser et al. 1988). Pooled epilimnetic water samples were incubated (4 hours at 19.5 C, dark) with 111 kBq of $NaH^{14}CO_3$; 3.5 μM NH_4Cl was added to the three enriched samples (no addition to controls). An initial sample was taken to account for any ^{14}C not incorporated into the cells. All samples were counted by liquid scintillation and the activity of the initial was subtracted from the final samples. The ammonium enhancement response was calculated as a t-statistic: Ammonium enhancement response = $(A_N A_C)/SE$, where A_N and A_C are the mean activities in the enriched and control samples, respectively, and SE is the pooled standard error of the mean (Box et al. 1978). The ammonium enhancement response becomes larger as the difference between enriched and control bottles increases, indicating greater nitrogen limitation.

Nutrient enrichment bioassays were conducted biweekly on pooled epilimnetic samples from each lake. Zooplankton were screened from the water using a 125 μm Nitex net. The design was a duplicated 2 x 2 factorial of nitrogen and phosphorus enrichments, using additions of 16 μM KH_2PO_4 and 160 μM NH_4Cl (Elser et al. 1988). These concentrations exceeded nutrient limiting conditions throughout the four-day bioassay (Bergquist, 1985). Samples were incubated at ambient lake temperature under 200 u-Einst $m^{-2}s^{-1}$ PAR on a 14:10 light/dark cycle for four days. Flasks were stoppered with sterile cotton. The samples were then filtered and analyzed for chlorophyll *a*. To determine the effects of the enrichments, the ratio of mean treatment chlorophyll to mean control chlorophyll was calculated for treatments with nitrogen alone, phosphorus alone, and nitrogen plus phosphorus.

Measurements of total nitrogen and total phosphorus were performed on pooled epilimnetic samples taken each week that were preserved by acidification and refrigeration of total nitrogen and freezing total phosphorus, and analyzed within six months of collection. The phenolhypochlorite method after micro-Kjeldahl digestion was used for total nitrogen, and the phosphomolybdate method after persulfate digestion was used for total phosphorus (Wetzel and Likens, 1979). All TN/TP ratios were calculated on a weight to weight basis.

Weekly zooplankton samples were taken using duplicate vertical hauls with a 80 μm mesh Nitex net and preserved in 4 percent sugared, buffered formalin. Crustacean herbivores were identified, counted, and measured, and biomasses were estimated as detailed by Carpenter et al. (1987).

Randomized intervention analysis tested for changes in the manipulated lakes, Peter and Tuesday, relative to the reference lake, Paul (Carpenter et al. 1989). Randomized intervention analysis, using series of parallel observations of experimental and reference ecosystems, paired in time, calculates a time series of interecosystem differences. The test statistic (T) is the absolute value of the difference between the pre-manipulation mean and the post-manipulation mean of the interlake differences. To determine whether a non-random change occurred in the manipulated lake, 1,000 random permutations of the interlake differences are performed and the proportion of these producing T values at least as large as the observed are determined. A low proportion (P) indicates that a non-random change occurred in the lake following the manipulation. Extensive testing has validated the use of randomized intervention analysis for whole-lake experiments (Carpenter et al. 1989). Autocorrelation in the time series of interlake differences biases random intervention analysis and can cause misleading results when the P value is between 0.01 and 0.05 (Carpenter et al. 1989). Only one time series of differences (nitrogen alone, Peter versus Paul lakes) had significant autocorrelation and P between 0.01 and 0.05. In that case, the effects of autocorrelation were determined by Monte Carlo simulation with an AR(1) model (Carpenter et al. 1989).

Results

Nutrient Dynamics

■ **Peter Lake.** Herbivore biomass (primarily the cladocerans *Daphnia pulex*, *D. rosea*, and *Holopedium gibberum*) increased significantly ($p < 0.001$) following the manipulation. However, herbivore length remained the same ($p = 0.747$), reflecting little change in species composition. Chlorophyll increased after the manipulation, although the response was transient and not significant ($p = 0.055$). Phyto-plankton biomass indicated by changing chlorophyll *a* concentrations in Peter Lake (Fig. 1B) increased substantially for approximately six weeks, but failed to remain high in the following two years. The gelatinous, green alga *Sphaerocystis schroeteri* was mainly responsible for the transient increase (Carpenter et al. 1987). The reference system, Paul Lake, reflected normal interannual variation for these lakes (Fig. 1A). For more detailed information concerning the manipulations, see Carpenter et al. (1987) and Carpenter and Kitchell (1988).

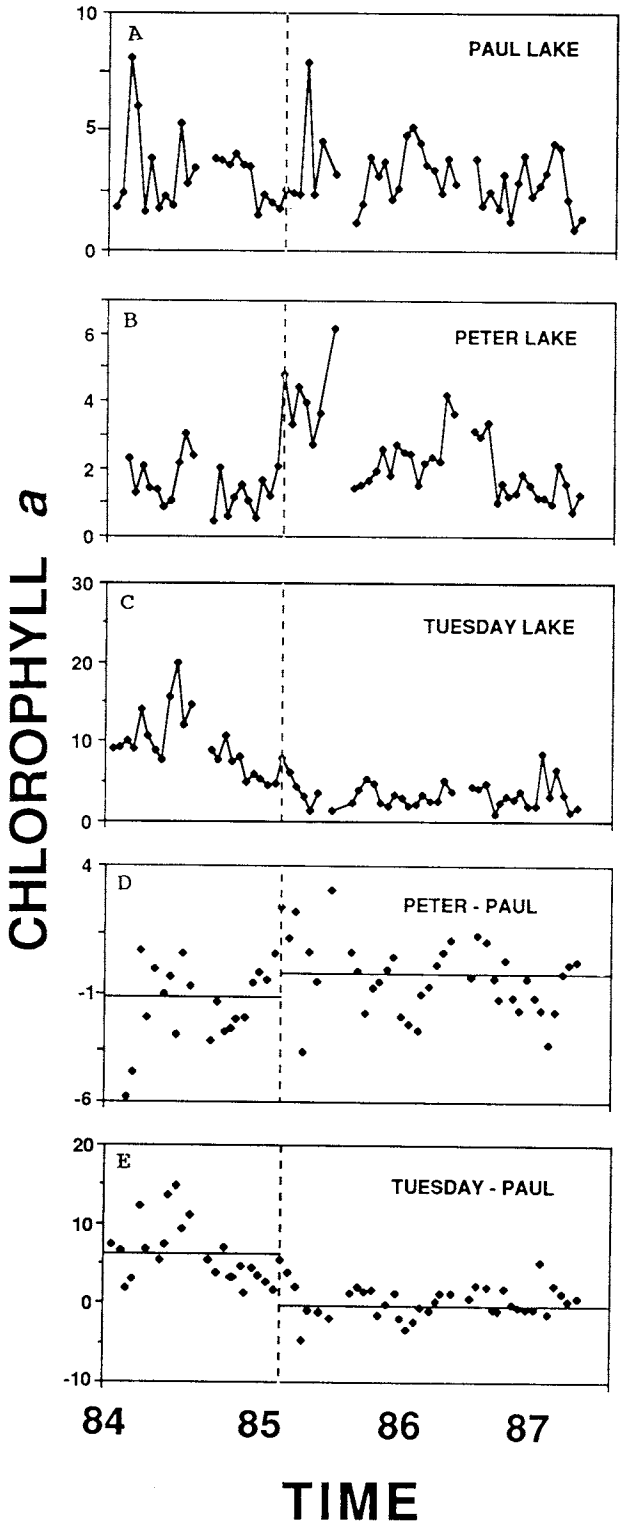

Figure 1.—Chlorophyll a (μg/L), 1984-1987. Sampling was conducted from mid-May through mid-September. A, B, C indicate time series for Paul, Peter and Tuesday Lakes, respectively. D and E are time series of differences between the manipulated lake and the reference lake. The vertical dashed line indicates the manipulation, Julian day 213, 1985.

Randomized intervention analysis showed that the phosphorus enrichment bioassay decreased significantly (Fig. 2B, $p = 0.005$), indicating less limitation by phosphorus. The phosphorus enrichment bioassay was consistently lower and less variable following the manipulation (Fig. 2D). The significance level of the phosphorus enrichment bioassay markedly increased ($D = -2.716$, $p < 0.0001$) if an aberrant data point on Julian day 155 in 1987 (Paul Lake) was not considered. Specific alkaline phosphatase activity (Fig. 3B) also decreased significantly, indicating less limitation by phosphorus ($p = 0.029$). The response, though, was not as strong as that in the nutrient enrichment (Table 1). Total phosphorus did not significantly change after the manipulation ($p = 0.415$).

The nitrogen enrichment bioassay (Fig. 4B) also increased significantly ($p = 0.043$), even after effects of autocorrelation were considered, indicating greater limitation by nitrogen (Table 1). Ammonium enhancement response showed a significant response to the manipulation ($p = 0.017$), demonstrating an increase in nitrogen deficiency (Fig. 5D). Neither total nitrogen ($p = 0.217$) nor total nitrogen/total phosphorus ($p = 0.217$) changed significantly with the manipulation. Interestingly, the combined enrichment (nitrogen plus phosphorus enrichment bioassay) did not respond to the manipulation ($p = 0.314$). The nitrogen plus phosphorus enrichment bioassay was always large (2-10X) compared to phosphorus enrichment bioassay or nitrogen enrichment bioassay, which reflects the co-limiting nature of nitrogen and phosphorus in these lakes (Elser et al. 1988).

■ **Tuesday Lake.** With the addition of a piscivore, herbivore biomass increased substantially ($p < 0.0001$). This shift to larger cladocerans (mostly *Daphnia pulex* and *Holopedium gibberum*) increased both biomass and herbivore length ($p < 0.001$). Phytoplankton biomass decreased significantly in the presence of large grazers ($p < 0.0001$). The algal assemblage shifted from large thecate dinoflagellates (*Peridinium* spp.) to small phytoflagellates, including *Cryptomonas* spp. and *Mallamonas* spp. (Carpenter et al. 1987). Chlorophyll *a* concentrations in Tuesday Lake remained low for the following two years (see Fig. 1E,C). More detailed information concerning the manipulations is available in Carpenter et al. (1987) and Carpenter and Kitchell (1988).

The phosphorus enrichment bioassay did not respond significantly to the manipulation ($p = 0.507$); however, if an aberrant data point from Paul Lake (Julian day 155, 1987) was removed, the significance level increased markedly ($D = 0.870$, $p = 0.078$).

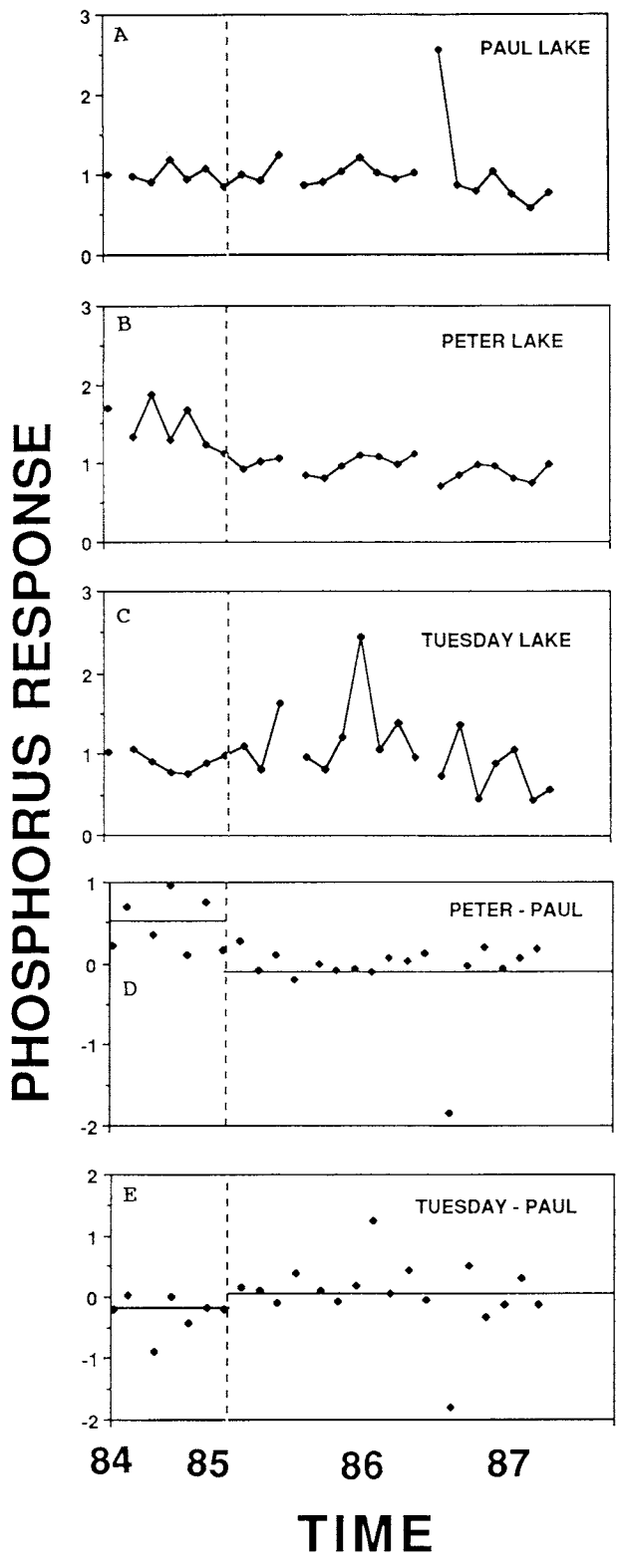

Figure 2.—Phosphorus response from the nutrient enrichment bioassays (treatment/control), 1984-1987. Sampling was conducted from mid-May through mid-September. A, B, C indicate time series for Paul, Peter and Tuesday Lakes, respectively. D and E are time series of differences between the manipulated lake and the reference lake. The vertical dashed line indicates the manipulation, Julian day 213, 1985.

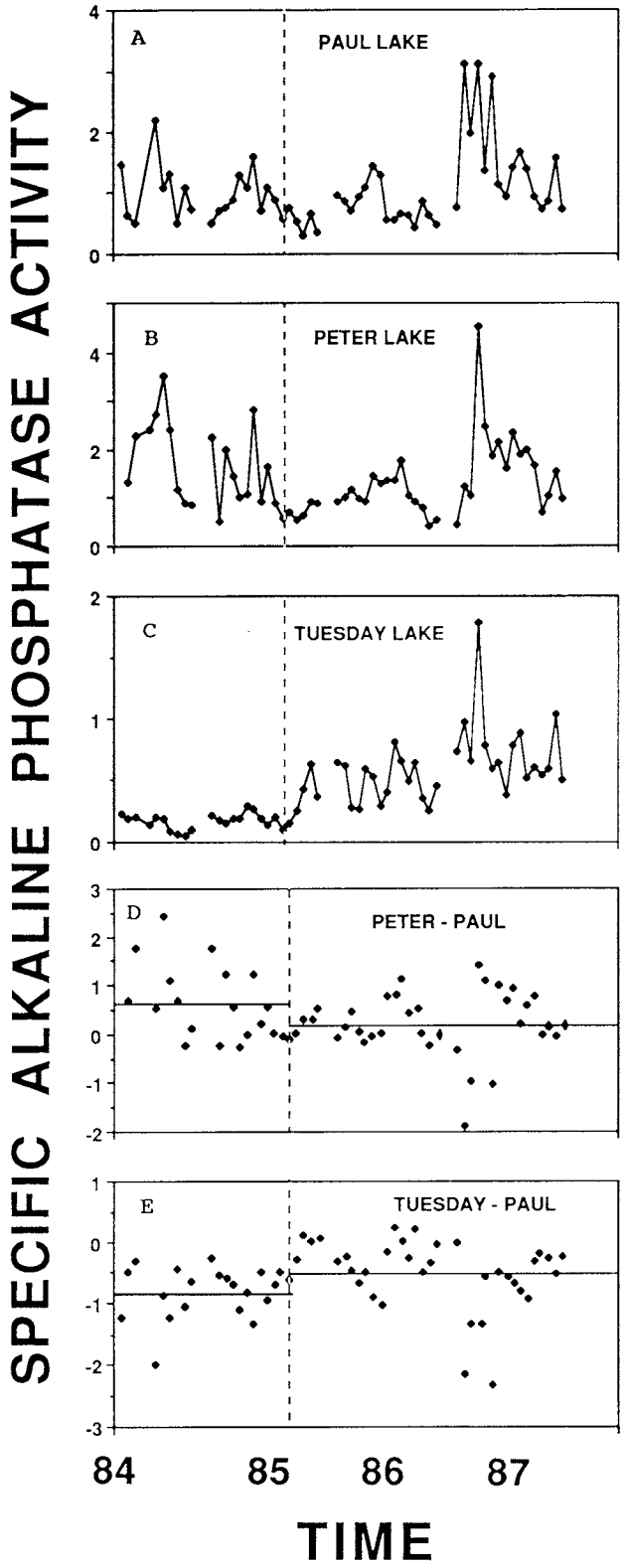

Figure 3.—Specific alkaline phosphatase activity (nmol PO_4^{3-} (μg Chl a)$^{-1}$ min^{-1}), 1984-1987. Sampling was conducted from mid-May through mid-September. A, B, C indicate time series for Paul, Peter and Tuesday Lakes, respectively. D and E are time series of differences between the manipulated lake and the reference lake. The vertical dashed line indicates the manipulation, Julian day 213, 1985.

Table 1.—Summary of randomized intervention analysis. Differences are expressed as (pre-manipulation mean−post-manipulation mean)/standard deviation.

ANALYSIS	DIFFERENCE	SIGNIFICANCE
CHLOROPHYLL		
Peter vs. Paul	0.591	n.s.
Tuesday vs. Paul	−2.120	***
HERBIVORE BIOMASS		
Peter vs. Paul	1.320	***
Tuesday vs. Paul	1.870	***
HERBIVORE LENGTH		
Peter vs. Paul	0.110	n.s.
Tuesday vs. Paul	2.640	***
P RESPONSE		
Peter vs. Paul	−1.336	**
Tuesday vs. Paul	0.151	n.s.
N RESPONSE		
Peter vs. Paul	1.420	*
Tuesday vs. Paul	−1.047	n.s.
N + P RESPONSE		
Peter vs. Paul	0.482	n.s.
Tuesday vs. Paul	0.125	n.s.
SPECIFIC APA		
Peter vs. Paul	−0.623	*
Tuesday vs. Paul	0.588	n.s.
AER		
Peter vs. Paul	0.707	*
Tuesday vs. Paul	−1.120	***
TP		
Peter vs. Paul	−0.220	n.s.
Tuesday vs. Paul	0.243	n.s.
TN		
Peter vs. Paul	0.331	n.s.
Tuesday vs. Paul	−0.077	n.s.
TN/TP		
Peter vs. Paul	0.340	n.s.
Tuesday vs. Paul	0.144	n.s.

n.s.−$p > 0.05$
*−$0.01 < p < 0.05$
**−$0.001 < p < 0.01$
***−$p < 0.001$.
Julian day 213, 1985 was considered the manipulation date.

Specific alkaline phosphatase activity (Fig. 4C) was not significantly different following the manipulation ($p = 0.073$), consistent with the results from phosphorus enrichment bioassay. Total phosphorus remained unchanged ($p = 0.358$).

The nitrogen enrichment bioassay gradually decreased following the manipulation, although not significantly ($p = 0.064$). As in Peter Lake, the nitrogen enrichment bioassay time series was autocorrelated. Although the direction of change was consistent between the nitrogen enrichment bioassay and the ammonium enhancement response, the ammonium enhancement response decreased much

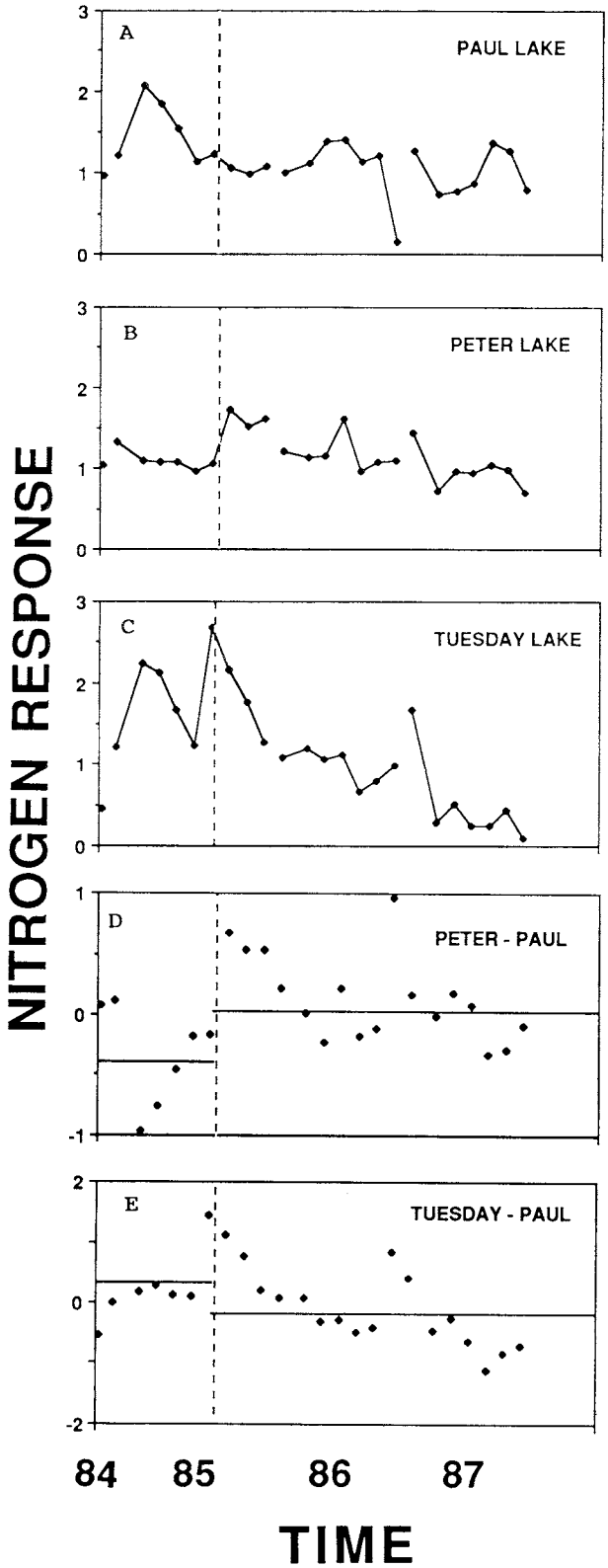

Figure 4.—Nitrogen response from the nutrient enrichment bioassays (treatment/control), 1984-1987. Sampling was conducted from mid-May through mid-September. A, B, C indicate time series for Paul, Peter and Tuesday Lakes, respectively. D and E are time series of differences between the manipulated lake and the reference lake. The vertical dashed line indicates the manipulation, Julian day 213, 1985.

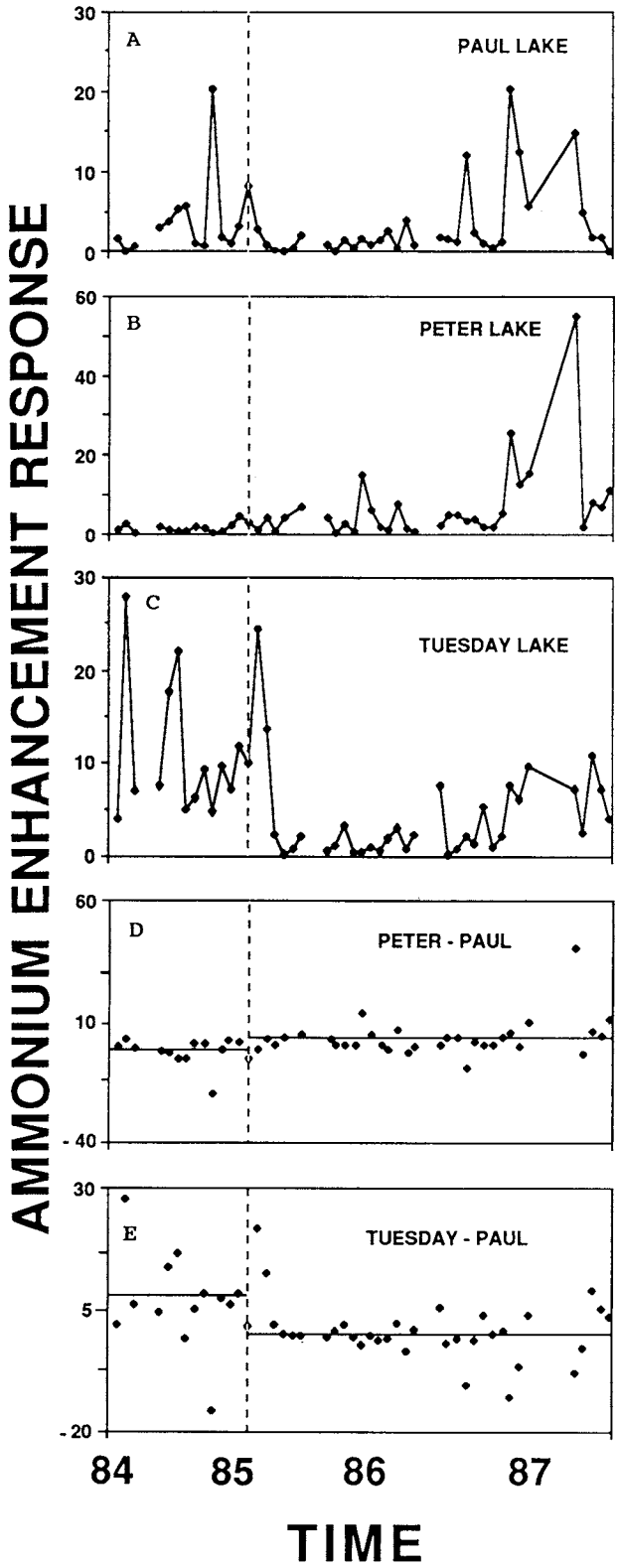

Figure 5.—Ammonium enhancement response, 1984-1987. Sampling was conducted from mid-May through mid-September. A, B, C indicate time-series for Paul, Peter and Tuesday Lakes, respectively. D and E are time series of differences between the manipulated lake and the reference lake. The vertical dashed line indicates the manipulation, Julian day 213, 1985.

more abruptly and significantly ($p < 0.0001$) after the manipulation (Table 1). Neither total nitrogen ($p = 0.789$) nor total nitrogen/total phosphorus ($p = 0.671$) changed significantly following the manipulation ($p = 0.789$). As in Peter Lake, the nitrogen plus phosphorus enhancement bioassay showed no response to manipulation (Table 1), although nitrogen phosphorus enhancement bioassay was always larger (2-10X) than either the phosphorus enhancement bioassay or the nitrogen enhancement bioassay (Elser et al. 1988).

Discussion

Several authors have distinguished nutrient limitation (population growth in the presence of nutrients) from nutrient deficiency (shift in cell metabolism) (Healey, 1973, 1979; Healey and Hendzel, 1980; Yentsch et al. 1977). Questions that address different scales are emphasized if one were to assess limitation within the current population (physiological indicators) versus assessing limitation based on assays that allow changes in the species composition (nutrient enrichments) (Howarth, 1988). O'Brien and Denoyelles (1974) and Gerhart and Likens (1975) suggest that despite methodological problems, nutrient enrichments accurately described the relationship between nutrient conditions and potential algal growth. Elser et al. (1988) also suggest that nutrient enrichments were a useful, but less sensitive complement to deficiency indicators.

Enrichment bioassays and physiological indicators indicated similar nutrient dynamics following the manipulation. In Peter Lake, phosphorus enrichment bioassay and specific alkaline phosphatase activity showed a decrease in phosphorus limitation while nitrogen enrichment bioassay and ammonium enhancement response reflected an increase in nitrogen limitation. Results were also consistent in Tuesday Lake. The phosphorus enrichment bioassays and specific alkaline phosphatase activity increased as nitrogen enrichment bioassay and ammonium enhancement response decreased. In Tuesday Lake, gradual but consistent changes in nitrogen enrichment bioassay were not significant. The ammonium enhancement response declined more abruptly following the manipulation, producing a significant result and reflecting the ammonium enhancement response's greater sensitivity to nutrient stress. Time series of nitrogen enrichment bioassays were autocorrelated in both Peter and Tuesday Lakes, while time series of physiological indicators were never autocorrelated.

Some of the discrepancy in sensitivity of measurements derives from species-specific responses to the availability of nutrients (Elrifi and Turpin, 1987). Vincent (1981) and Healey and Hendzel (1976) found that the presence of heterocystic blue-green algae caused spuriously high ammonium enhancement response results, regardless of nitrogen deficiency. Addition of ammonium to *Anabaena* spp. can completely suppress nitrogen fixation (Takahashi and Saijo, 1988), which might be a factor in the atypical ammonium enhancement response of heterocystic blue-greens. In our study lakes, heterocystic blue-greens rarely dominated the assemblage and did not appear to affect the results of ammonium enhancement response. However, in lakes where heterocystic or nitrogen-fixing blue-greens dominate, more than one physiological indicator of nitrogen limitation would be useful (Healey, 1975, 1979). Healey and Hendzel (1979) also found variability in the response of *Cryptomonas erosa* to several indicators of phosphorus deficiency, again suggesting caution in interpreting results from any one measure of population or cellular nutrient limitation (Goldman and Dennett, 1983, 1986).

Conclusions

Nutrient enrichment bioassays and physiological nutrient deficiency indicators led to the same conclusions regarding ecosystem change in two manipulated lakes. Ammonium enhancement response was more sensitive than nitrogen enrichment bioassays. Both enrichment bioassays and physiological indicators were more sensitive to ecosystem change than total nutrient concentrations. In contrast to the nutrient enrichment time series, physiological indicator time series were never autocorrelated. Physiological indicators measure the extant algal assemblage, are rapidly assayed, and can be run more frequently than nutrient enrichments. Ideally, multiple indicators of nutrient availability are desirable (Goldman and Dennett, 1983, 1986; Healey, 1975; Healey and Hendzel, 1976, 1980). However, when resources limit the number of variables that can be determined, physiological indicators appear to be more useful indicators of ecosystem response than enrichment bioassays.

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