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Limnology and Oceanography, Vol. 33, No. 1 (Jan., 1988), 1-14.

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LIMNOLOGY AND OCEANOGRAPHY

January 1988

Volume 33

Number 1

Limnol. Oceanogr., 33(1), 1988, 1-14
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Zooplankton-mediated transitions between N- and P-limited algal growth¹

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Abstract

Limitation of algal growth by nitrogen and phosphorus was assessed in three north-temperate lakes with physiological bioassays and nutrient enrichment experiments. In addition, mesocosm experiments were performed in the three lakes to examine the effects of nutrient enrichment and zooplankton biomass and size on algal nutrient status. In situ indicators of N and P availability were inversely related in magnitude and transitions between N and P limitation were abrupt. Physiological bioassay results did not indicate simultaneous limitation by N and P. However, limited responses to single-nutrient enrichment and pronounced responses to simultaneous N and P addition in enrichment experiments suggested that potential limitation by the secondary nutrient was usually in close proximity to limitation by the primary nutrient. Transitions between N and P limitation closely accompanied major shifts in the zooplankton community. The importance of the zooplankton community in regulating the relative degree of N or P limitation was confirmed by the mesocosm experiments, which demonstrated that transitions between algal N or P limitation could be induced by manipulations of zooplankton biomass or size. This result supports a hierarchical view of the function of planktonic systems, in which biotic interactions structure the response of the algal community to a given nutrient load.

Phosphorus and nitrogen commonly limit algal growth in lakes and oceans. In freshwaters, phosphorus is the most frequent limiting factor (Schindler 1977), although transitions between phosphorus and nitrogen limitation often occur seasonally and in anthropogenically eutrophic lakes (Wetzel 1983). Further evidence for the importance of nitrogen as a growth-limiting factor in freshwater is provided by both experimental and correlational studies (Goldman 1972; White et al. 1982; Canfield 1983; Vincent

et al. 1984; and others) and by synergistic effects of simultaneous N and P enrichment on algal growth in nutrient bioassays (Fuhs et al. 1972; Gerhart 1975; Elser and Kimmel 1985; and others). Transitions between P and N limitation can determine the outcome of interspecific competition (Rhee and Gotham 1980; Tilman 1982) and, in single-species culture experiments, conform to Liebig's law of the minimum (Rhee 1978).

Less is known about how mixed communities undergo shifts in P and N limitation in lakes poor in both nutrients. We used two approaches to evaluate N and P limitation in three nutrient-poor lakes: physiological bioassays (for P limitation, alkaline phosphatase activity; Pettersson 1980; for N limitation, NH_4^+ enhancement of dark carbon uptake; Yentsch et al. 1977) and laboratory nutrient enrichment exper-

¹ A contribution (funded by NSF grants BSR 83-08918 and BSR 86-06271) from the University of Notre Dame Environmental Research Center.

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iments performed with the natural lake assemblages. The combined use of physiological assays and conventional enrichment experiments may provide for a more comprehensive assessment of an algal community's nutritional status (Healey and Hendzel 1980; Elser and Kimmel 1986).

During the stratified season in many lakes, nutrient recycling by zooplankton can satisfy the nutrient requirements of a rapidly growing algal assemblage (Lehman 1980) and can significantly alter its nutritional status (Bergquist and Carpenter 1986; J. J. Elser et al. 1986). Furthermore, differential recycling of N and P, dependent on zooplankton species composition, nutritional status, and food quality, may influence the relative degree of N or P limitation of algal growth by altering the range of resource ratios experienced by algal species with different nutrient affinities (Lehman 1984). However, no field data document zooplankton-mediated shifts between phosphorus- and nitrogen-limited algal growth. In this paper, we present such evidence and demonstrate that, where ambient nutrient conditions are suitable, the biomass and composition of the zooplankton community can strongly affect algal nutrient limitation.

We thank J. Kitchell and J. Hodgson for their contributions to the fish manipulations in these lakes, N. Goff and P. Soranno for technical assistance, and D. Lodge, P. Richerson, J. Lehman, and three anonymous reviewers for comments on the manuscript.

Materials and methods

Study sites—Paul, Peter, and Tuesday lakes are small (surface area, 0.8–2.4 ha), relatively deep (maximum depth, 12–19 m) kettle basins located within 0.5 km of each other within the boundaries of the University of Notre Dame's Environmental Research Center, section 36, T45N, R42W, Gogebic County, Michigan. Additional limnological information is given elsewhere (Carpenter et al. 1986). The lakes have a history of whole-lake experimentation (Schmitz 1958; M. M. Elser et al. 1986) and are presently the objects of whole-lake fish manipulation experiments. Major changes in the zooplankton communities in Peter

and Tuesday lakes reported here resulted from the manipulations of the fish communities of those lakes (Carpenter et al. 1987).

Routine monitoring—We monitored various physical, chemical, and biological properties at a permanent midlake station in each lake at weekly intervals between 20 May and 18 September 1985. Sampling was confined to the period of stable stratification to minimize the potentially confounding effects of vertical mixing on nutrient availability and phytoplankton growth.

Zooplankton samples were collected by duplicate vertical hauls through the water column with a 80- μm -mesh Nitex net. The duplicate samples were pooled, preserved in 70% ethanol, and the animals later counted, identified, and measured. Zooplankton lengths were subsequently converted to masses with the empirical equations of Downing and Rigler (1984), except for *Holopedium gibberum*, for which the equation of Peters and Downing (1984) was used. Biomass-weighted average zooplankton mass for the zooplankton community (ZM_B , $\mu\text{g animal}^{-1}$) was calculated for each sampling day as:

$$ZM_B = [\sum (I_i \times B_i)] / \sum B_i$$

where I_i is the average body size of species i on that day ($\mu\text{g animal}^{-1}$) and B_i is the total biomass of species i present on that day ($\mu\text{g liter}^{-1}$). Further details regarding this calculation are given elsewhere (Elser et al. 1987).

We took water samples from the depths of 100, 50, 30, 10, 5, and 1% light penetration with an opaque, horizontal Van Dorn bottle. Water from each depth was analyzed for chlorophyll concentration (corrected for pheopigments) by fluorometry (Strickland and Parsons 1968) after extraction of the filtered material in methanol with a sonicating homogenizer and alkaline phosphatase activity (APA) after the method of Pettersson (1980). The mean mixed-layer chlorophyll-specific APA ($\text{SpAPA} = \text{APA}/\text{Chl}$) was used as an indicator of phosphorus deficiency.

Water from the three epilimnetic depths was pooled and used for several subsequent analyses. NH_4^+ enhancement of dark ^{14}C

uptake was measured according to the method of Yentsch et al. (1977), with opaque bottles (300 ml), ^{14}C inoculations of 296 kBq (8 μCi), and 50 μM spikes of $\text{NH}_3\text{-N}$. Bottles were incubated in the dark at 19.5°C for 4.5–5 h, after which the contents were filtered onto GF/F glass-fiber filters. The NH_4^+ enhancement of dark ^{14}C -uptake response (AER) was calculated as:

$$\text{AER} = (\text{dpm}_N - \text{dpm}_0) / (\text{dpm}_C - \text{dpm}_0),$$

where dpm_N is the mean radioactivity of NH_4^+ -enriched bottles, dpm_C is the mean radioactivity of control bottles, and dpm_0 is the radioactivity of time-zero controls. Treatments were generally performed in triplicate, but duplicates were used for the biomass experiments. Student's *t*-tests ($P = 0.05$) were used to test for significant differences between enriched- and control-bottle radioactivity.

Mixed-layer samples for analysis of total phosphorus (TP, phosphomolybdate method after persulfate digestion: Wetzel and Likens 1979) and total nitrogen (TN, phenol-hypochlorite method after micro-Kjeldahl digestion: Wetzel and Likens 1979) were preserved by freezing (TP) and acidification and refrigeration (TN) and analyzed within 6 months of collection.

Enrichment experiments—Nutrient enrichment bioassays were performed at bi-weekly intervals on pooled mixed-layer water samples. Zooplankton were removed by screening samples through 125- μm Nitex netting; this procedure removed little algal biomass (mean chlorophyll decrease after screening: 5.3%). The following enrichments were made in duplicate: +16 μM P, +160 μM N, +16 μM P and +160 μM N, and control (no addition), using 300 ml of sample in 350-ml Erlenmeyer flasks. Enrichments were made as aqueous solutions of KH_2PO_4 and NH_4Cl . Enrichments with iron-EDTA (3.75 μM Fe as FeCl_3) and silica (32 μM Si as Na_2SiO_3) never resulted in discernible responses, and therefore we consider N and P enrichments only. The flasks were incubated at ambient lake temperature (light intensity: 200 $\mu\text{Einst m}^{-2} \text{ s}^{-1}$, 14:10 L/D) for 4 d, after which the contents of each flask were filtered and analyzed for chlorophyll *a* concentration. Results of the

bioassays were analyzed by ANOVA and significant differences ($P = 0.05$) between treatments were evaluated with Tukey's multiple comparison test (SAS Institute 1985).

Field experiments—In summer 1986, two mesocosm experiments were performed to evaluate relationships between zooplankton biomass (biomass experiment) and size structure (size experiment) and relative N or P limitation of algal growth. Mesocosms consisted of 45-liter plastic buckets mounted in a floating plastic frame. Screening over the top of each mesocosm reduced incident light intensity by about 50%. The mesocosms were filled by pumping water from a depth of 0.75 m through 125- μm -mesh screen to remove zooplankton. This procedure eliminates >90% of the zooplankton (Bergquist 1985) while removing <15% of the algae. Each experiment proceeded for 3 d, and the mesocosms were thoroughly mixed daily. All treatments were assigned randomly to mesocosms.

Experiments to evaluate the effect of zooplankton biomass on algal nutrient limitation were conducted in all three lakes simultaneously, starting on 19 June 1986. On the preceding night, zooplankton were collected from each lake with 5-m vertical hauls of an 80- μm -mesh Nitex net. The animals were acclimated to surface temperatures overnight. Dead or injured animals floating at the surface and *Chaoborus* larvae were removed by hand. Zooplankton were added to mesocosms to form a gradient of zooplankton biomass: 0.25 x , 0.5 x , 0.75 x , 1 x , 2 x , 4 x , and 8 x , where x = ambient density. A single mesocosm was used at each zooplankton density (except 1 x , see below). To eliminate the possible effect of nutrients that may have accumulated in the zooplankton stock due to overnight excretion, we gently rinsed the zooplankton twice before adding them to the mesocosms. Two additional treatments were performed at the 1 x density. Two mesocosms received a nutrient enrichment (+16 μM $\text{PO}_4^{3-}\text{-P}$ and +160 μM $\text{NH}_4^+\text{-N}$) in addition to a 1 x addition of zooplankton. To evaluate whether nutrients present in the zooplankton stock (but not removed by the rinsing procedure) or in the guts of the zooplankton contributed

to these observations, we made both a 1x addition of live zooplankton and a 1x addition of heat-killed zooplankton to one mesocosm.

A sample was taken of the zooplankton stock to determine the actual biomass added to the mesocosms. Before adding the zooplankton, water samples for chlorophyll, APA, and AER analysis were taken. After 3 d, samples for chlorophyll, APA, and AER analyses were taken along with a sample of the final zooplankton community (9-liter Schindler trap) for enumeration and sizing. Live samples were also taken to evaluate *Daphnia* and *Holopedium* survivorship, which was relatively high (>75%) and did not vary appreciably among zooplankton densities. Average zooplankton biomass in each 1x mesocosm was calculated on the basis of the counts of the initial zooplankton stock and of the final zooplankton sample.

On 21 August 1986, an experiment to evaluate the effect of the size distribution of the zooplankton community (at constant total zooplankton biomass) on algal nutrient status was begun in Paul Lake with Tuesday Lake zooplankton. The zooplankton were collected from Tuesday Lake for two reasons: the community was relatively simple on this date (dominated by *Daphnia* and *Holopedium* with few other taxa present), thus simplifying interpretation of the results, and, the zooplankton in Tuesday Lake did not migrate vertically, so that the full size range of zooplankton could be collected with horizontal hauls at the surface on the morning of the experiment. This collection procedure prevented appreciable mortality after collection and guaranteed that vertically migrating *Chaoborus* larvae were not present in the zooplankton.

Zooplankton were collected with horizontal hauls at a depth of 0.5 m with 80- μm -mesh Nitex nets. The animals were fractionated into large and small fractions after each haul by pouring the contents of the plankton bucket into a fractionator consisting of a 0.3-m length of 5-cm PVC pipe with a piece of 700- μm -mesh Nitex netting mounted in the middle. The fractionator was suspended in a bucket of surface lake water so that the screen was submerged, but the bottom of the pipe was well above the

bottom of the container. Smaller zooplankton passed through the screen, while larger animals were retained in the pipe above the screen and poured off into another container. This procedure resulted in little mortality and injury to the zooplankton and divided them into two distinctly sized communities of about equal biomass.

After the animals were collected and fractionated, the dry weight concentration of each fraction was determined. Appropriate amounts of each fraction were then added to duplicate mesocosms to achieve a total biomass equivalent to ambient zooplankton density (estimated by dry-weight determination of the pooled contents of duplicate 9-liter Schindler trap samples at 0, 1.5, and 3 m taken in Paul Lake two nights before the experiment) but in a mixture of small and large animals in the following percentages: 100% large, 75% large, 50% large, 25% large, and 0% large. Each zooplankton fraction was rinsed as described above, and a sample of each was preserved for enumeration and measurement. Samples for chlorophyll, APA, and AER analyses were taken before zooplankton were added.

Chlorophyll, APA, and AER analyses were performed for each mesocosm. Additionally, a zooplankton sample from one duplicate of each zooplankton mixture was taken (9-liter Schindler trap) for counting and sizing to evaluate the final biomass and size distribution achieved in the experiment. Examination of samples before preservation indicated that survival of *Daphnia* and *Holopedium* was high (>80%) and did not differ systematically between treatments. The mean zooplankton biomass in each treatment did not vary appreciably (<20%) or systematically between size treatments. Both mesocosms at 75% large zooplankton were contaminated by an unidentified source of nutrients and were excluded from these analyses.

Results

In situ patterns in 1985—Epilimnetic algal biomass was highest in Tuesday Lake during the study until the final weeks of the study period, when chlorophyll declined dramatically as large-bodied cladocerans appeared in the lake (Carpenter et al. 1987).

Table 1. Atomic N:P ratios and chlorophyll concentrations ($\mu\text{g liter}^{-1}$) in the mixed layers of Paul, Peter, and Tuesday lakes during summer stratification, 1985. Means with SD in parentheses are presented. In all cases, $n = 16$.

	Paul	Peter	Tuesday
N:P	11.0 (4.9)	13.3 (3.3)	11.2 (3.7)
Chl	3.26 (1.53)	2.47 (1.72)	5.50 (2.65)

For the most part, chlorophyll concentrations in Peter Lake were the lowest of the three lakes (Table 1). The atomic ratio of TN to TP varied between 4.5 and 27.5 in the three lakes (Table 1).

Cladocerans (*Daphnia pulex*, *Holopedium gibberum*, and *Daphnia rosea*) dominated the Paul and Peter lake zooplankton communities, although in Peter Lake, cladoceran biomass decreased considerably af-

ter day 64 (Fig. 1A, B). In Tuesday Lake, small omnivorous copepods (primarily *Tropocyclops prasinus*) dominated the zooplankton biomass until day 79, after which cladoceran biomass (mainly *H. gibberum* and *D. pulex*) increased dramatically and copepod biomass declined (Fig. 1C). Rotifers were a very small component of the zooplankton biomass in all three lakes (Fig. 1).

Average zooplankton mass was relatively stable in Paul Lake (Fig. 1A). In Peter Lake, ZM_B declined substantially after day 64, reflecting a decline in *Daphnia* biomass (Fig. 1B). Tuesday Lake zooplankters were extremely small until day 79, when ZM_B increased substantially as large-bodied cladocerans appeared in large numbers.

Physiological indicators of N and P limitation behaved in a complementary fashion in all three lakes (Fig. 2). In Paul Lake, when SpAPA was maximal (days 28–42), AER

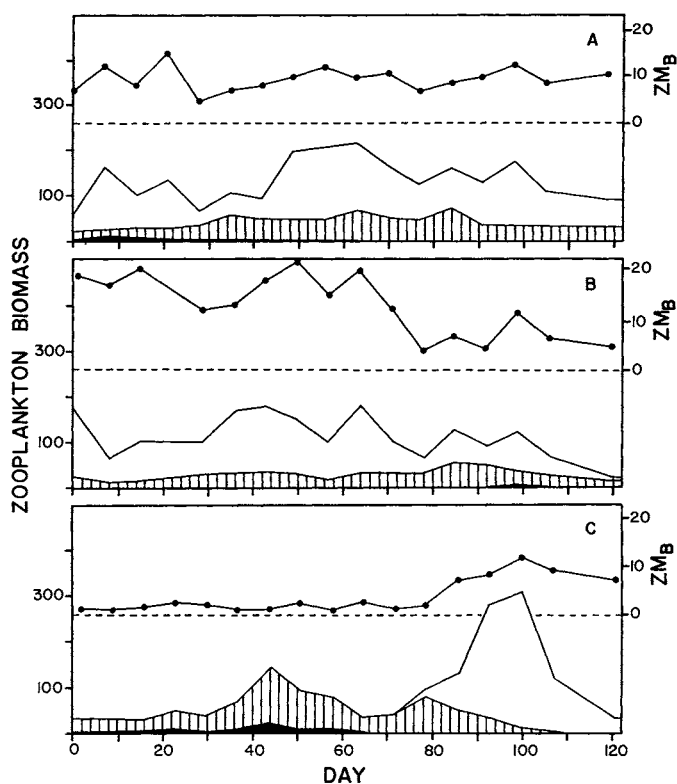


Fig. 1. Biomass-weighted average zooplankton size (ZM_B , $\mu\text{g animal}^{-1}$) (top) and zooplankton biomass (units $\mu\text{g liter}^{-1}$) (bottom) of cladocerans (blank), omnivorous copepods (striped), and rotifers (black) for Paul Lake (A), Peter Lake (B), and Tuesday Lake (C). Day 1 = 20 May 1985.

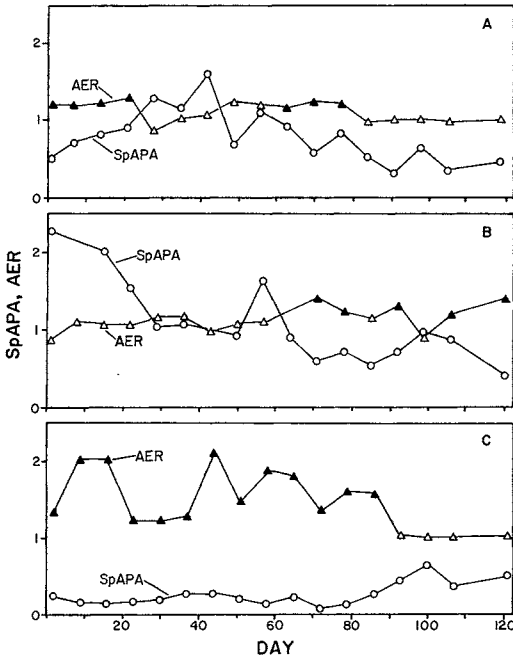


Fig. 2. Mean mixed-layer, chlorophyll-specific, alkaline phosphatase activity [O—SpAPA, nmol PO_4^{3-} ($\mu\text{g Chl } a$) $^{-1}$ min^{-1}] and NH_4^+ enhancement of dark ^{14}C uptake response (Δ —AER, dimensionless) for Paul Lake (A), Peter Lake (B), and Tuesday Lake (C). Statistically significant AER— \blacktriangle . Day 1 = 20 May 1985.

decreased and became nonsignificant (Fig. 2A). The subsequent decline in SpAPA was accompanied by a return of AER to significant levels. In Peter Lake, SpAPA was relatively high, but declining, throughout the early sampling season until day 69, when it reached a minimum value and AER rose and became significant (Fig. 2B). In Tuesday Lake, AER was high, significant, and variable during the first 86 d of the sampling season (Fig. 2C). SpAPA was extremely low, but rose substantially after day 86, when AER decreased and became nonsignificant.

The results of the nutrient enrichment experiments provide further indications of the dynamics of N and P limitation in these lakes (Fig. 3). Statistically significant responses to phosphorus added alone were rare (Fig. 3: Paul and Tuesday lakes, day 118; Peter Lake, day 17). Enrichment by nitrogen alone led to significant increases in algal growth in Tuesday Lake in all experiments but three, but in only one experiment in Peter and Paul lakes (Fig. 3). How-

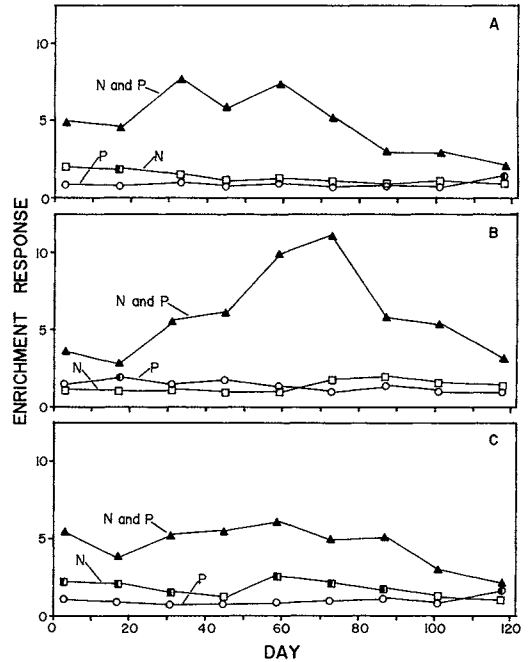


Fig. 3. Responses to experimental enrichment (treatment chlorophyll/control) for Paul Lake (A), Peter Lake (B), and Tuesday Lake (C). Open symbols signify treatments not significantly different from the control. Treatments statistically distinguishable from the control are closed; those that differ from each other as well are shown \blacksquare and \bullet . Day 1 = 20 May 1985.

ever, transitions between P and N limitation may still be examined from trends in the magnitudes of responses. In Paul Lake, nitrogen was of greater importance as a limiting factor early in the season (Fig. 3A). However, between days 33 and 118, control, phosphorus, and nitrogen treatments were virtually indistinguishable. In Peter Lake, P-response and N-reponse levels switched around day 60 (Fig. 3B), suggesting that the primary limiting nutrient was P before day 60 and N after day 60. In Tuesday Lake, N was clearly of primary importance until day 101, with P the primary limiting factor on day 118 (Fig. 3C). Algal growth in flasks enriched simultaneously with N and P was always significantly greater than that in control flasks and in flasks enriched with P or N alone (Fig. 3).

The temporal patterns suggested by the physiological bioassays and nutrient enrichment experiments are generally consistent

Table 2. Summary of linear regression analyses for nutrient, algal, and zooplankton parameters. Data for AER:SpAPA and N response:P response were log-transformed before regression with ZM_B .

Dependent variable	Independent variable	P	R ²	Slope
a. P response	SpAPA	0.016	0.23	0.61
b. N response	AER	0.003	0.29	0.38
c. AER:SpAPA	TN:TP	0.06	0.07	-0.51
d. N resp.:P resp.	TN:TP	0.006	0.26	-0.14
e. AER:SpAPA	Total zoopl. biom.	0.001	0.21	-0.02
f. N resp.:P resp.	Total zoopl. biom.	0.11	0.10	-0.003
g. AER:SpAPA	ZM_B	0.0001	0.58	-0.12
h. N resp.:P resp.	ZM_B	0.0001	0.57	-0.06
i. SpAPA	ZM_B	0.0001	0.44	0.07
j. AER	ZM_B	0.0001	0.34	-0.03
k. P response	ZM_B	0.0005	0.39	0.03
l. N response	ZM_B	0.0002	0.42	-0.05
m. TN:TP	ZM_B	0.01	0.13	0.11

in each lake (Figs. 2, 3). However, linear regressions of P response with SpAPA and N response with AER were statistically significant but relatively weak (Table 2 a, b: $P < 0.02$, $R^2 < 0.30$). This lack of quantitative correspondence between the two methodologies resulted primarily from instances when the physiological indicator was elevated but there was little or no response to single nutrient enrichment (e.g. SpAPA and P response: Paul Lake, days 27–35, Tuesday Lake, day 101; AER and N response: Paul Lake, days 59–73).

Both the physiological bioassay and nutrient enrichment results clearly indicate P and N limitation to be inversely related in these lakes (Fig. 4). High levels of SpAPA occurred only when AER was low (Fig. 4A). Likewise, appreciable response to a single nutrient enrichment generally corresponded to a negligible response to the other (Fig. 4B). Transitions between N and P limitation occurred within 1 week and the phytoplankton community rarely showed signs of both N and P limitation.

The ratio of the N-limitation indicator to the P-limitation indicator on a given day for both physiological assays and enrichment experiments (i.e. AER:SpAPA or N response:P response) was used to examine transitions between periods of dominantly N- or P-limited algal growth. The limitation ratios were only weakly related to atomic TN:TP in the lakes (Table 2 c, d), although examination of the data indicated that primarily N-limited algal growth occurred only below TN:TP of about 15:1. This transi-

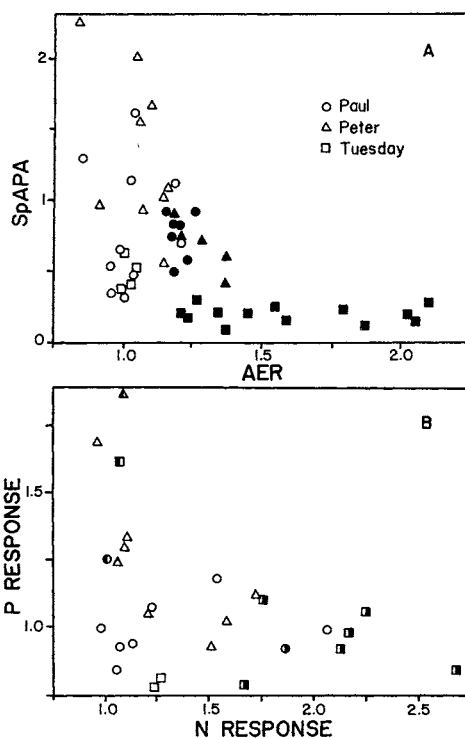


Fig. 4. A. Mean mixed-layer, chlorophyll-specific, alkaline phosphatase activity [SpAPA, units $\text{nmol PO}_4^{3-} (\mu\text{g Chl } a)^{-1} \text{ min}^{-1}$] vs. NH_4^+ enhancement of dark ^{14}C uptake response (AER, relative units). Closed symbols indicate days when AER was statistically significantly different from zero. B. Phosphorus response (P-enriched chlorophyll/control chlorophyll) in a given experiment vs. nitrogen response (N-enriched chlorophyll/control chlorophyll) in that experiment. Statistically significant responses to P enrichment— \bullet , \blacktriangle ; significant N responses— \blacksquare , \bullet .

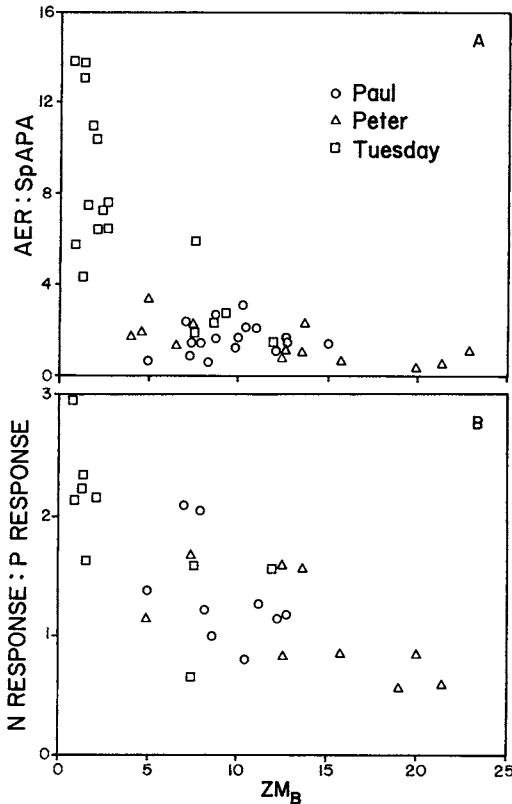


Fig. 5. AER:SpAPA (A) and N response:P response (B) vs. ZM_B .

tion ratio corresponds well to the Redfield ratio (15: Redfield 1958) and to the average optimal N:P ratio for various freshwater algae found by Rhee and Gotham (1980).

The relative degree of N or P limitation was evaluated in relation to both total zooplankton biomass and biomass-weighted average zooplankton mass (ZM_B). N limitation was dominant only at low zooplankton biomass, with nutrient limitation shifting toward P limitation when zooplankton were abundant, although these relationships were also relatively weak (Table 2 e, f). However, when limitation ratios were related to ZM_B , much clearer relationships resulted (Fig. 5, Table 2 g, h). As the zooplankton community was dominated by larger animals, the phytoplankton community became P limited. The effect of the zooplankton community on relative N or P limitation is particularly obvious from the responses in Tuesday Lake: the abrupt qual-

itative and quantitative shift in the zooplankton community after day 79 (Fig. 1) was accompanied by a drastic change from algal growth that was strongly N limited to growth more limited by availability of P (Figs. 2, 3). This transition is reflected by the relationship between the limitation ratios and ZM_B in Tuesday Lake (Fig. 5). The strong association between the limitation ratios and ZM_B reflects opposite linear relationships between the individual limitation indicators and ZM_B : phosphorus indicators were positively related to ZM_B and nitrogen indicators were negatively related to ZM_B (Table 2 i-l). Further contributing to these observations is a positive correlation between TN:TP and ZM_B (Table 2 m).

Algal responses in mesocosms— ZM_B values of the zooplankton used in the biomass experiments in the three lakes were quite similar (10.7 – $12.6 \mu\text{g animal}^{-1}$), and all three communities were dominated by large-bodied cladocerans (66–98%), predominantly *Daphnia*. However, the biomass ranges used in the three lakes differed substantially (22 – $710 \mu\text{g liter}^{-1}$ in Paul, 9.6 – $307 \mu\text{g liter}^{-1}$ in Peter, and 34 – $1,090 \mu\text{g liter}^{-1}$ in Tuesday), due to differences in densities of ambient zooplankton.

The responses of the algal communities of Paul, Peter, and Tuesday lakes to experimental manipulation of zooplankton biomass and nutrient availability were similar (Figs. 6–8). Chlorophyll declined significantly ($P < 0.05$, $R^2 > 0.64$) with increasing zooplankton biomass in all three (Figs. 6A, 7A, and 8A). However, the overall change in chlorophyll was not large (21–46% decline in chlorophyll across the 32-fold range of zooplankton biomass)—similar to previous experiments in these lakes which evaluated total algal biovolume across gradients of zooplankton biomass (Elser et al. 1987). Responses to N and P enrichment were pronounced in all three lakes: chlorophyll in the enriched mesocosms was 2.8–3.1 times higher than in the control mesocosm, indicating that the algal communities were highly nutrient limited (Figs. 6A, 7A, and 8A). In Paul and Tuesday lakes, chlorophyll levels in the mesocosm to which dead zooplankton were added were not appreciably different from the control, indi-

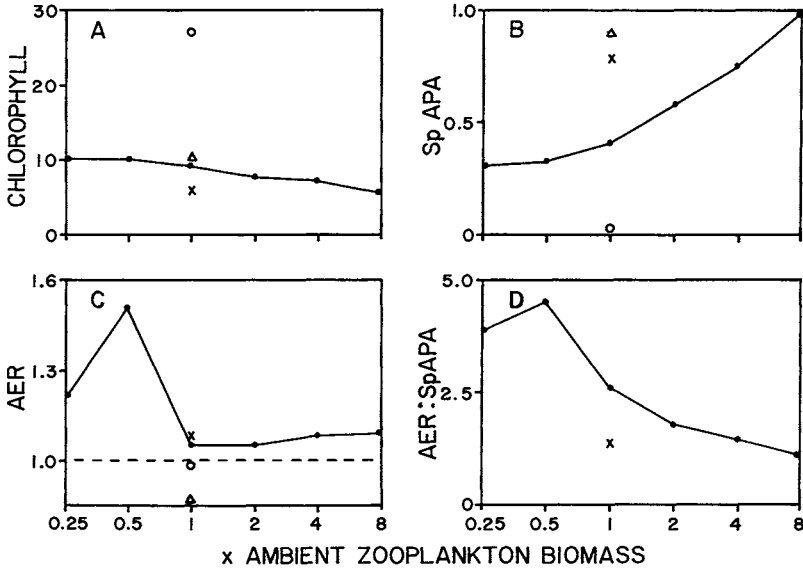


Fig. 6. Results of biomass experiment in Paul Lake. A. Chlorophyll concentration ($\mu\text{g liter}^{-1}$). B. Specific APA [$\text{nmol PO}_4^{3-} (\mu\text{g Chl } a)^{-1} \text{ min}^{-1}$]. C. AER (dimensionless). D. AER:SpAPA vs. level of zooplankton biomass (multiples of 1x ambient density). Unenriched mesocosms—●; mean of duplicate, N+P-enriched mesocosms—○; mesocosm with 1x live zooplankton and 1x heat-killed zooplankton—Δ; initial value—x. Note logarithmic scale of zooplankton axis.

cating that nutrients added with the zooplankton stock were not a factor in these experiments. This was not true in Peter Lake, however, so that the data for this lake should be interpreted with caution.

The patterns between algal nutrient limitation and zooplankton biomass were consistent with field observations and were similar in the experiments in all three lakes (Figs. 6B, C, 7B, C, and 8B, C). Specifically,

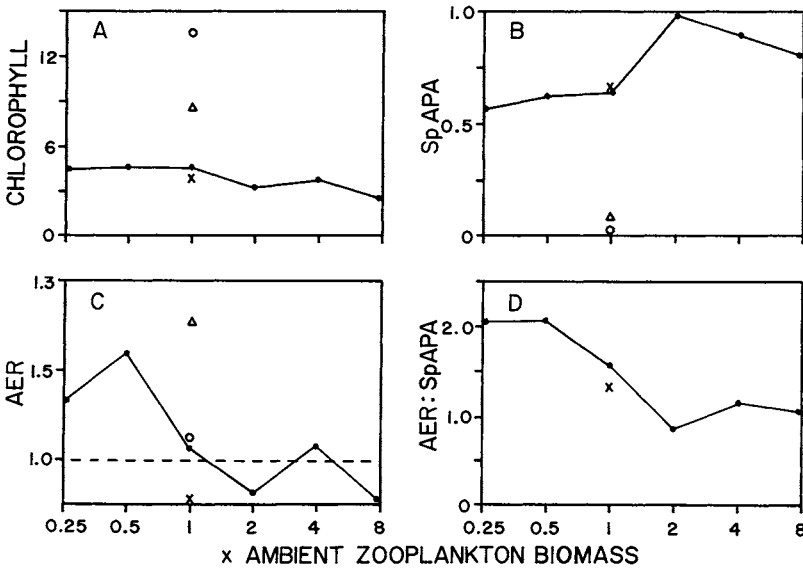


Fig. 7. As for Fig. 6, but for Peter Lake.

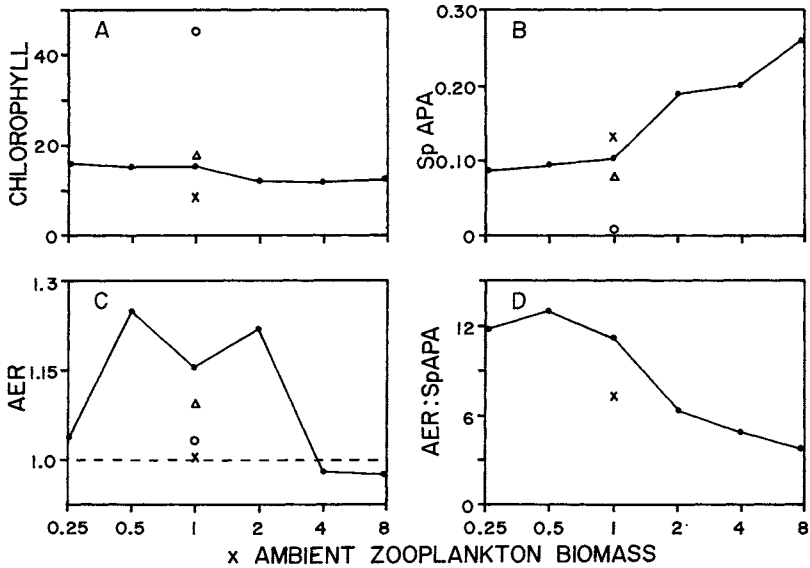


Fig. 8. As for Fig. 6, but for Tuesday Lake.

SpAPA increased substantially with increasing zooplankton biomass. This increase was significant in Paul and Tuesday lakes ($P < 0.001$, $R^2 > 0.96$), but not in Peter Lake (although SpAPA was consistently higher at biomass levels $> 1x$). The less substantial and nonsignificant increase in SpAPA in Peter Lake probably reflects the effects of residual P that were present in the zooplankton stock, as suggested by the chlorophyll and SpAPA values in the mesocosm that received dead zooplankton (Fig. 7A, B). The increase in SpAPA in Paul and Tuesday lakes reflects both a significant increase in total APA ($P < 0.01$, $R^2 > 0.90$) and the decrease in chlorophyll with increasing zooplankton. SpAPA in the enriched mesocosms was extremely low compared to the unenriched (Figs. 6B, 7B, and 8B), indicating that the bioassay was quite responsive to availability of P.

Nitrogen limitation (as indicated by AER) decreased with increasing zooplankton biomass in the experiments in all three lakes (Figs. 6C, 7C, and 8C). Due to the abrupt switches from high to low values of AER, linear regressions of AER with zooplankton biomass were not significant. However, consistent trends of low AER at high zooplankton biomass are evident in all three experiments, even though they do not fit a

simple statistical model. AER values in enriched enclosures were always low and ~ 1.0 , indicating that the assay was sensitive to availability of N. In the mesocosms that received dead zooplankton in Paul and Tuesday lakes, AER was lower compared to the $1x$ control enclosure; however, it is difficult to interpret relatively small differences given the variability in AER that we observed. In Peter Lake, AER in the mesocosm that received dead zooplankton was elevated substantially (Fig. 8C); it may reflect the addition of P with the dead zooplankton, which probably accentuated the degree of N limitation.

As a result of the increases in SpAPA and decreases in AER with increasing zooplankton biomass, the ratio of AER:SpAPA (an indicator of the balance between N or P limitation) decreased extremely strongly and significantly ($P < 0.03$, $R^2 > 0.75$) with zooplankton biomass in all three lakes (Figs. 6D, 7D, and 8D). This result corroborates the field observations in these lakes, indicating that zooplankton can have a substantial effect on the balance between N and P limitation of algal growth.

In the size experiment, mean ZM_B (geometric mean of initial and final ZM_B of the mesocosm zooplankton) ranged from 8.3 to $14.1 \mu\text{g animal}^{-1}$ across the size distribution

treatments. Zooplankton size had a strong effect on relative N or P limitation and the observed effects were consistent with observations made for the algal communities in situ in 1985. Chlorophyll decreased weakly ($\sim 20\%$) with zooplankton size (Fig. 9A); however, this trend was not statistically significant ($P = 0.23$, $R^2 = 0.59$). SpAPA increased strongly with zooplankton size (Fig. 9B: $P = 0.02$, $R^2 = 0.95$), while AER was a strong negative function of ZM_B ($P = 0.04$, $R^2 = 0.92$), and no individual AER determinations were significant in the presence of the larger zooplankton (Fig. 9B). The ratio AER:SpAPA declined substantially with increasing zooplankton size (Fig. 9C: $P = 0.03$, $R^2 = 0.93$), indicating that the phytoplankton were predominantly N limited when grazed by small zooplankton, but were P limited when grazed by larger animals, consistent with field observations made in 1985.

Discussion

Physiological bioassays and nutrient enrichment experiments yielded similar patterns of N and P limitation in situ in these lakes in 1985 (Figs. 2 and 3) and indicated similar relationships between algal nutrient limitation and possible controlling factors (Table 2, Fig. 5). However, the quantitative correspondence between the two methods was poor because of modest growth responses to single nutrient addition that occurred even when the physiological assay indicated that the algae were highly deficient in that nutrient. This result suggests that conventional nutrient enrichment experiments alone are a less sensitive means of assessing algal nutritional status in lakes where two (or more) nutrients are present in relative concentrations near the Redfield ratio. Addition of the primary limiting nutrient may increase algal growth slightly, but transition to limitation by the alternate nutrient may be so rapid that no response is detectable. This finding suggests that physiological assays will more reliably indicate the duration and degree of deficiency of the nutrient primarily limiting to the dominant taxa in the assemblage, as well as more closely reflect in situ conditions of nutrient supply (Elser and Kimmel 1986).

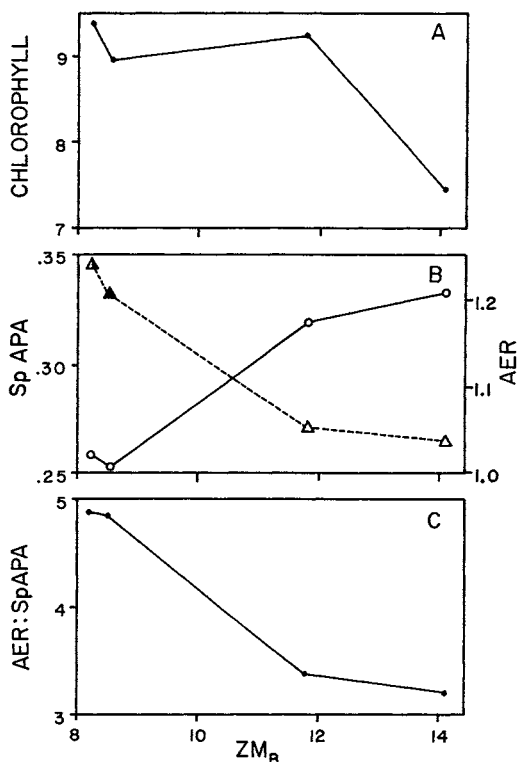


Fig. 9. Results of the zooplankton size experiment. Chlorophyll concentration ($\mu\text{g liter}^{-1}$) (A), SpAPA [$\text{nmol PO}_4^{3-} (\mu\text{g Chl } a)^{-1} \text{min}^{-1}$] and AER (Δ —dimensionless) (B), and AER:SpAPA (C) vs. ZM_B ($\mu\text{g animal}^{-1}$). Shading of AER symbol indicates whether both (\blacktriangle), and one (\triangle), or neither (\triangle) AER determination(s) was significant in the duplicate mesocosms for that treatment.

The abrupt changes in indicators of N or P limitation that we observed, both in situ and in mesocosms, probably reflect both physiological responses by the dominant algae present and compositional shifts induced by selective grazing or nutrient competition in regimes with differing ratios of resource supply (sensu Tilman 1982). Species composition of the algal communities in the three lakes was dynamic and many changes were associated with changes in the zooplankton community (Elser et al. 1987; Elser and Carpenter 1988). Species shifts in mesocosm experiments of this sort in these lakes, however, have been relatively subtle (Bergquist 1985; Bergquist and Carpenter 1986; Elser et al. 1987) and suggest that the pronounced effects observed in these mesocosm experiments reflect physiologi-

cal responses of the existing algal taxa. Whether the observed transitions reflect physiological responses of existing algal taxa or competitively induced shifts in composition between species with differing physiological properties or growth requirements is secondary to the primary effect of zooplankton on relative nutrient recycling. A possible confounding factor is selective grazing of algal species on the basis of relative N and P requirements. However, no evidence is available to evaluate this possibility.

Two important implications for future study of algal community ecology arise from these discussions: the interpretation of supposed competitive interactions across gradients of resource supply will be complicated by the possibility of selective grazing if the gradients are influenced by zooplankton nutrient recycling; and, studies of the species-specific responses of algae to zooplankton will be confounded by the effect of zooplankton on relative availability of N and P, as differing responses of algal species will not reflect simply their relative susceptibility to grazing but potentially their relative N and P growth requirements as well.

The specific mechanisms responsible for zooplankton-mediated shifts between N- and P-limited algal growth cannot be inferred from these data, although several possibilities are suggested. The simplest mechanism is one in which the N:P ratio of zooplankton nutritional requirements is generally lower than that for algae; the zooplankton would then inherently tend to recycle N in greater amounts relative to P, which would be expressed as changes in relative availability of N and P as either zooplankton biomass or size distribution changed (due to allometric effects). However, zooplankton could well have the same N:P requirements as algae. But if the N:P ratio of excreta exceeded that of egesta, recycling would be higher for N than for P, as the products of excretion are available for uptake immediately while the availability of egested nutrients may be delayed. Given the complex relationships between N and P recycling by zooplankton and the nutritional status of their food, the nutritional status of the animals themselves (Lehman

and Naumoski 1985; Olsen and Ostgaard 1985), and zooplankton species and size (Peters 1975; Lehman 1980), the possibilities for future investigation are considerable.

Changes in the zooplankton communities of Peter and Tuesday lakes that led to changes in the nature of algal nutrient limitation resulted from experimental manipulations of the fish populations of these lakes (Carpenter et al. 1987). Observations from other lakes also suggest that food-web interactions can play a role in regulating relative availability of N and P. In Round Lake, Minnesota (Shapiro and Wright 1984), fish manipulations induced the appearance of large-bodied cladocerans. The mixed-layer TN:TP ratio increased because of reductions in TP greater than those of TN. Shapiro and Wright attributed the general decrease in nutrients to the effects of vertically migrating *Daphnia* (Wright and Shapiro 1984) but did not specifically discuss the changes in N:P. In Lake Trummen, Sweden, trophic structure was invoked to explain a discrepancy between epilimnetic TN:TP and the dominance of blue-greens in a year following a winter fishkill (Smith 1983). These observations and the results reported here indicate that changes in food-web structure can alter ecosystem nutrient recycling in unexpected ways.

From the dynamics of algal growth and nutrient limitation in these lakes, we conclude that biotic interactions structure the response of the algal community to a given nutrient load. In Paul, Peter, and Tuesday lakes, the N:P supply ratio is such that subtle biotic interactions (such as differential nutrient recycling by zooplankton) can determine which nutrient primarily limits algal growth. In other lakes, however, internal and external loading may provide one nutrient in such excess relative to algal demand that the effects of zooplankton on relative nutrient availability would be insignificant. However, given the considerable variability in published phosphorus loading-chlorophyll relationships (Shapiro 1979), the observation that some variation in the TP-chlorophyll relationship can be attributed to aspects of the zooplankton community (Hrbáček et al. 1978; Pace 1984)

and the dynamics of zooplankton, algae, and nutrients following experimentally or naturally induced changes in lake food webs (Schindler and Comita 1972; Andersson et al. 1978; Henrikson et al. 1980; Shapiro and Wright 1984, this study), further study of food-web effects on phytoplankton-nutrient interactions is warranted.

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Submitted: 7 December 1986

Accepted: 10 April 1987

Revised: 9 October 1987