Ecological and nutritional studies on *Dinobryon* Ehrenb.: Seasonal periodicity and the phosphate toxicity problem

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Abstract

Although field studies show inverse correlations between concentrations of dissolved phosphate and abundances of *Dinobryon*, phosphorus is not toxic even at high concentrations. Species abundance in water of low phosphate concentration may be traced instead to effective mechanisms for phosphate uptake which allow the cells to thrive at low ambient levels of PO_4-P. Half-saturation constants of less than 0.5 μM PO_4-P can be demonstrated both in laboratory cultures and in situ in lakes. Ambient concentrations of potassium ion can be toxic to a variety of species of *Dinobryon* and thus can limit both their geographical and seasonal occurrence. Ammonium serves as a better nitrogen source than does nitrate, an observation supported by both field and laboratory data. At least one species, *Dinobryon sertularia*, is physiologically limited to water temperatures below 20°C.

The widespread occurrence of the chrysophyte *Dinobryon* and its characteristic seasonal periodicity, particularly in oligotrophic waters, have prompted repeated efforts to link its dominance and decline to environmental factors. Pearsall (1932) and Hutchinson (1944) were among the first to correlate the occurrence of *Dinobryon divergens* with periods of high nitrate: phosphate ratios, or simply of low PO_4-P in natural waters. Rodhe's (1948) demonstration that minute additions of potassium phosphate to Lake Erken water were lethal to that species seemed to be conclusive proof of phosphate inhibition, but subsequent studies have shown that luxurious growth of *Dinobryon* species can occasionally be found in water with high phosphate concentrations and that enrichment of natural waters or unialgal cultures with phosphate can sometimes enhance growth, even of *D. divergens* (Lefèvre and Farrugia 1958; Talling 1962; Munch 1972).

Other aspects of *Dinobryon* ecology, while not as contradictory as are its phosphate requirements, are largely considered on the basis of field observations alone and have not been subjected to rigorous experimental tests. The species occur most often in oligotrophic waters of neutral or slightly acid pH, but *D. divergens* (Hamilton 1969) and *Dinobryon* sp. (Tressler and Domogalla 1931) have been observed in waters as alkaline as pH 8.7. A similar latitude exists with regard to thermal optima. Hutchinson (1944) reported population maxima of *D. divergens* at 16.5–23.5°C, Vetter (1937) at 6.2°C, and Dimitz (1938) at temperatures <7.6°C. In another species, *Dinobryon sertularia*, ecotypic variation with temperature does not appear to be as great, and species maxima generally occur in cold water (G. W. Prescott personal communication). Many investigators, among them Asmund (1955) and Kozarov (*cited in* Stankovič 1960), have documented seasonal succession of several *Dinobryon* species, a phenomenon for which species-specific optima for temperature and light are sometimes proposed.

A study of plankton periodicity in a pond with *D. sertularia* as one of the dominant species showed that population densities of *Dinobryon* were inversely related to concentrations of phosphate and inorganic nitrogen, and they showed a dependence on water temperature as well. To interpret the field data in light of possible phosphate toxicity, I isolated several species of *Dinobryon* into defined mineral media.

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Methods

Field observations included weekly samples from October 1968 to June 1969 taken from a 3-m-deep artificial pond owned by the Boy Scouts of America in North Brunswick, New Jersey. All sampling was done from a station over the deepest point of the pond. Chemical measurements for surface samples were performed with a Hach chemical analysis kit, and volumetric samples for phytoplankton counts were taken from 0, 1, and 2.5 m with a Kemmerer sampler.

Specimens of *D. sertularia* were isolated from the net plankton of the pond in November 1970 and were initially maintained in DB1 (Provasoli unpublished), a medium that contains a large proportion of peat extract added to a mineral salt base. The cells were freed from bacteria by treating a growing culture with 4 mg of penicillin, 0.5 mg of streptomycin, and 0.01 mg each of the sulfa drugs gantrisin, sulfamerazine, sulfisoxazolum, homosulfamine, and sulfisomidine per milliliter final concentration, then making daily transfers to fresh media, and testing the sterility of each subculture. A completely defined medium was subsequently developed for the cells, and all experiments reported were performed in this medium. *Dinobryon cylindricum* and *Dinobryon sociale* var. *americanum* were isolated into it and maintained in unialgal culture. Laboratory stocks were initiated from several colonies of each species.

Most of the results reported here are based on experiments in screwcap Pyrex tubes containing 10 ml of culture medium. Cells were counted with a hemocytometer or Nageotte counting chamber from live samples. The cells settled to the bottoms of the chambers and swam very slowly, so that individual counts were replicable to within 5%. Unless otherwise stated, *D. sertularia* and *D. cylindricum* were incubated at 14°C on a 14L/10D regime, illuminated by "soft-white" fluorescent light at 0.02–0.04 ly min^{-1} [light intensities were measured in foot-candles and later converted to ly min^{-1} by Strickland's (1958) conversion factor: 1 fc = 6.5×10^{-5} ly min^{-1} for PHAR: 380–720 nm]; *D. sociale* var. *americanum* was grown at 20°C on a 16L/8D photoperiod. Cell yields were followed semiquantitatively throughout each experiment, but exhaustive counts were generally made only once, after about 6 weeks, when cell concentrations had stabilized to a maximum. For other than experiments requiring serial transfers, tubes were inoculated with 3–4 drops from stock cultures or directly from previous experiments. Three or

Table 1. Composition of *Dinobryon* culture medium DyIII.

	mg $liter^{-1}$
$CaCl_2 \cdot 2H_2O$	75
$NaSiO_3 \cdot 9H_2O$	15
$MgSO_4 \cdot 7H_2O$	50
MES* (2-[N-Morpholino] ethane Sulfonic Acid)	200
NH_4NO_3	5
$NaNO_3$	20
KCl	3
Sodium glycerophosphate	10
Na_2EDTA	8
Fe (electrolytic)†	0.7
Mn (as Cl)	0.2
Zn (as SO_4)	0.04
Mo (as Na_2MoO_4)	0.02
Co (as Cl)	0.008
B (as H_3BO_3)	0.8
Biotin	0.0005
Thiamine	0.2
B_{12}	0.0005
pH	6.8

*MES is available from Sigma Chemical Company.

†Fe (electrolytic) is prepared as a stock solution by adding a precise weight of electrolytic iron to sufficient HCl to dissolve it, and then diluting with distilled water to the final volume.

four replicates were usually prepared for each experimental treatment.

Cells were prepared for phosphate uptake experiments by washing growing cultures with phosphate-free culture medium (Table 1) and by adding subsamples of this suspension to medium containing concentrations of $^{31}P/^{33}P$-sodium phosphate of known specific activity. After incubation for 1 h (0.032 ly min^{-1}), during which the vials were agitated manually at 5-min intervals to reduce the local depletion of nutrients around cells (Fuhs et al. 1972), the cells were collected on Millipore filters presoaked in 1 mM PO_4-P, and washed with 4–6 ml of the growth medium. Experimental suspensions of cells were dilute enough so that usually <10% of the radioactive phosphorus was removed from solution during incubation. Placing two filters in series showed that by this method the membrane filter itself binds <1% of the total radioactive phosphorus that is retained by the cells. The filters were air-dried, added to 10 ml of toluene-Liquifluor (New England Nuclear Corp.), and sample activity was assessed by liquid scintillation counting. Uptake values are corrected for self-absorption and quenching.

Rates of uptake of phosphorus by lake plankton were measured by essentially the method just described, except that lake water from 2-m depth rather than a dilute laboratory culture was used as the incubation medium, and samples were incubated in situ for 1 h.

Results

The defined medium and cation effects—A preliminary, completely defined medium was prepared by compounding the average concentrations of anions present in the pond during exponential growth of *Dinobryon*, measured during the field study with the Hach chemical analysis kit, and the major cations, determined from emission spectroscopy of the pond water during a bloom of *D. sertularia*. The resulting solution, buffered with 200 mg $liter^{-1}$ MES (Sigma) at pH 6.8, permitted growth of *Dinobryon* as well as many other species from the pond. The cells remain colonial in culture, with *D. sertularia* having a minimum doubling time of about 2 days, whereas *D. cylindricum* and *D. sociale* var. *americanum* have doubling times of about 1 day. The buffer does not seem to have any toxic effects, as varying it from 100–500 mg $liter^{-1}$ does not depress final observed yields, but it does slow the division rates at higher concentrations. At lower concentrations, however, the buffer apparently does not maintain the pH during autoclaving, and a microscopic precipitate can be seen in the tubes afterwards.

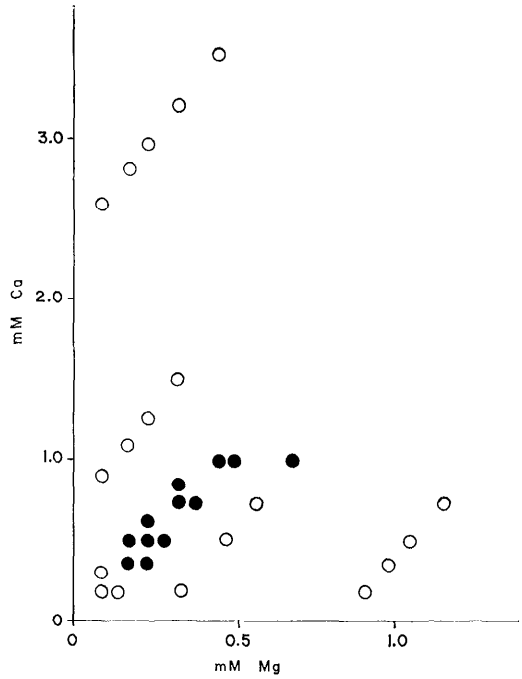

Fig. 1. Optimal Ca:Mg molar ratio in terms of cell yield for *Dinobryon sertularia* in DyIII culture medium. ○—Both tubes of the pair of experimental replicates had final yields of fewer than 5×10^3 cells ml^{-1}; ●—both replicates had greater than 50×10^3 cells ml^{-1}.

The artificial medium was improved by manipulation of the major cations. In one experiment the basal salt composition of the medium was prepared at five different concentrations (Fig. 1). Separate addition of calcium as calcium chloride or magnesium as magnesium sulfate greatly de-

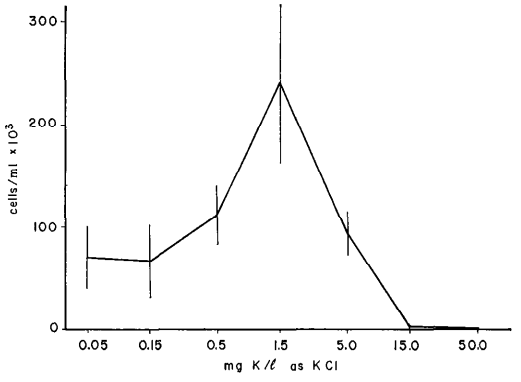

Fig. 2. Yield of *Dinobryon sertularia* after 6 weeks in DyIII medium as a function of potassium concentration. Plotted are means and 95% confidence limits for pooled replicate culture tubes. Analysis of variance discerned no valid differences among replicates ($p > 0.1$).

presses growth whenever the addition sufficiently alters the optimal Ca:Mg ratio, about 2:1 on a molar basis, or about 3.2:1 on a weight basis. A lower limit for total solids of about 50 ppm is apparent, since even optimal Ca:Mg ratios at that low level do not permit dense growth. In similar experiments, when NaCl was added to various concentrations of the medium, no toxicity was observed at chloride concentrations, and $Cl:SO_4$ ratios, equal to some of those which were lethal when $CaCl_2$ was the chloride source, implying that the earlier results were due to specific cation, rather than anion, effects. Because there must be an upper limit to the tolerance of any element, even if its effect is strictly osmotic, failure to find toxicity with NaCl simply means that the highest concentrations used (60 mg liter^{-1}) were below this upper limit.

Dinobryon sertularia tolerates a broad range of sodium concentrations, but its sensitivity to potassium, as Fig. 2 shows, is quite marked. Growth is almost totally inhibited at 15 mg liter^{-1} K, but even at 5 mg liter^{-1} growth is depressed. The toxicity is not absolute, but it can be heightened or alleviated, depending on the combined concentrations of calcium and magnesium. Dependence of potassium toxicity on diva-

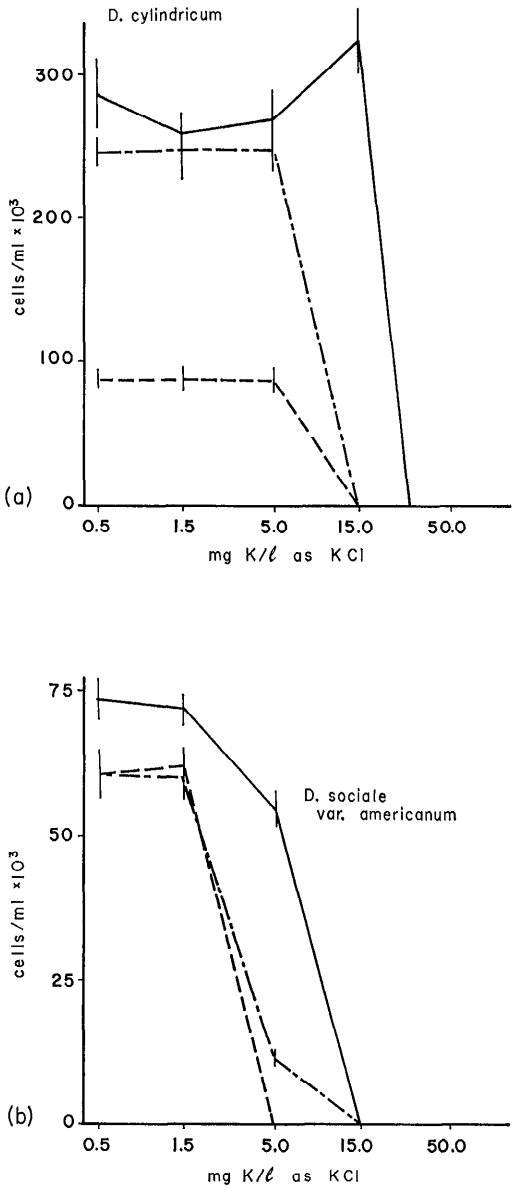

Fig. 3. Response of *Dinobryon cylindricum* (a) and *Dinobryon sociale* var. *americanum* (b) to potassium concentrations at various levels of calcium and magnesium: 0.1 mg liter^{-1} Ca, 0.025 mg liter^{-1} Mg (— — —); 1.0 mg liter^{-1} Ca, 0.25 mg liter^{-1} Mg (— — —); and 10.0 mg liter^{-1} Ca, 2.5 mg liter^{-1} Mg (———). Plotted are means and 95% confidence limits for pooled counts of 3–4 replicate culture tubes. Analysis of variance discerned no valid differences among replicates. The solid line in (a) intercepts the x-axis at 30 mg liter^{-1} K.

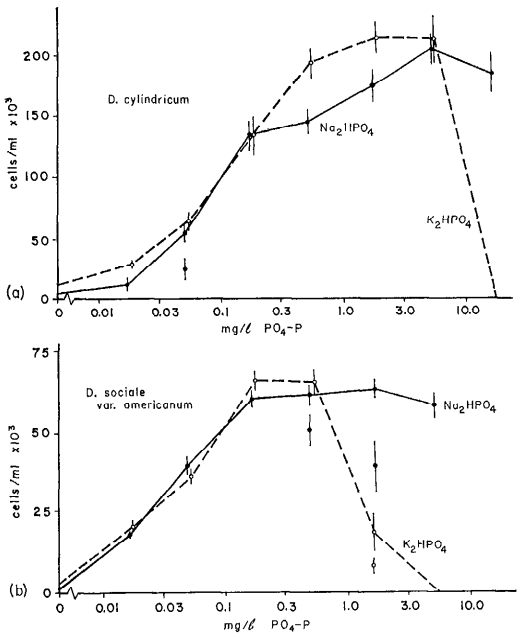

Fig. 4. Response of *Dinobryon cylindricum* (a) and *Dinobryon sociale* var. *americanum* (b) to phosphorus added either as Na_2HPO_4 or as K_2HPO_4. Plotted are means and 95% confidence limits for pooled counts of 3–4 replicate culture tubes. Means and confidence limits for counts of individual members of the series which analysis of variance showed to differ ($p < 0.05$) from the other replicates are plotted separately. The dashed lines intercept the x-axis at 15 mg liter^{-1} in (a), and at 6 mg liter^{-1} in (b).

lent cation concentration is shown in Figs. 3a and b for *D. cylindricum* and *D. sociale* var. *americanum* in unialgal culture. In both cases, *Dinobryon* species tolerate higher concentrations of potassium when levels of divalent cations, Ca and Mg, are increased. The greater tolerance of *D. cylindricum* to potassium at any given level of calcium and magnesium seems to be a species-specific difference and may be related to the overall superior performance of that species in the artificial medium.

Potassium toxicity is further manifest in Figs. 4a and b, which show yields of cultures with potassium or sodium phosphate as the phosphorus source. The toxic effects at high concentrations are related to potassium rather than phosphorus, as cultures

Table 2. Average composition of pond water the week before peak abundances of *Dinobryon*, compared to DyIII culture medium. Values are mg liter^{-1}, but Ca:Mg and K:(Ca + Mg) are molar ratios.

	Pond	Medium
Na	2.3	9.6
K	1.2	1.5
Ca	4.9	20.0
Mg	1.6	5.0
Cl	13.0	37.0
SO_4	22.0	20.0
SiO_2	1.4	3.3
Fe	0.45	0.7
NH_4-N	0.3	0.87
NO_3-N	0.02	4.18
PO_4-P	0.02	1.32
Ca:Mg	1.8	2.4
K:(Ca+Mg)	0.17	0.056
pH	6.6-7.2	6.8

supplied with sodium phosphate grow well even at high P concentrations. Furthermore, potassium concentrations of the cultures in the K_2HPO_4 series which show toxic effects are approximately those which cause toxicity at comparable levels of Ca and Mg when potassium is added as KCl (Figs. 3a and b). This evidence suggests that the ratio of potassium to divalent cations may be important in a general way, and Table 2 shows that the Ca:Mg and K:(Ca + Mg) molar ratios of the pond are similar to those of the best culture medium (Table 1) although the chemical compositions differ considerably.

Phosphate uptake kinetics—Field populations of *Dinobryon* showed four major peaks during the study. Throughout this period, concentrations of molybdate-reactive phosphate were near the limit of detection by the method used (below 0.1 mg P liter^{-1}). Nonetheless during maximal *Dinobryon* abundances, dissolved PO_4-P was always completely undetectable in the water, although the element was normally measurable in acid-hydrolyzed samples of lake water containing phytoplankton. Also an increase of *D. cylindricum* directly fol-

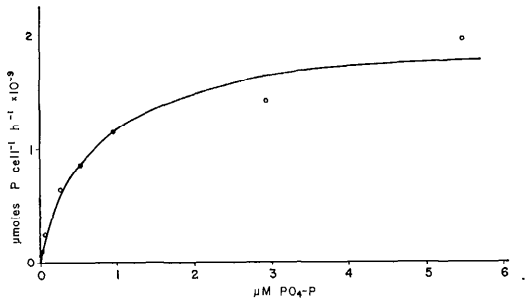

Fig. 5. Phosphorus uptake by *Dinobryon cylindricum*. The curve is the least-squares fit of a rectangular hyperbola to the data points. $k_s = 0.72$ µM P (SE = 0.17, df = 5); $u_m = 2.01 \times 10^{-9}$ µmoles cell^{-1} h^{-1} (SE = 0.07×10^{-9}).

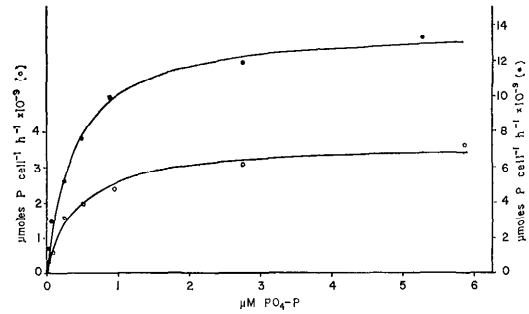

Fig. 7. Phosphorus uptake by *Dinobryon sociale* var. *americanum* held in P-free media for 2 days (upper curve), or taken directly from a growing culture (lower curve). Upper curve $k_s = 0.39$ µM P (SE = 0.03, df = 5), $u_m = 13.9 \times 10^{-9}$ µmoles cell^{-1} h^{-1} (SE = 0.03×10^{-8}); lower curve $k_s = 0.42$ (SE = 0.05, df = 5), $u_m = 3.66 \times 10^{-9}$ (SE = 0.14×10^{-9}).

lowed a bloom of *Peridinium*, and most of the population expansion by *Dinobryon* was achieved after phosphate had declined to undetectable levels. Part of this characteristic behavior by *Dinobryon* species may be explained by their kinetics of phosphate uptake, as *D. cylindricum* displays a half-saturation constant for uptake (k_s) of 0.72 µmoles P liter^{-1} (Fig. 5). *Peridinium*, on the other hand, has $k_s = 6.3$ µmoles P liter^{-1} (Fig. 6) and is thus much less adept at absorbing phosphorus from low ambient concentrations. Curves have been fit to the data points by nonlinear regression, using the equation

$$u = u_m S/(k_s + S), \qquad (1)$$

where u is the rate of uptake of phosphate,

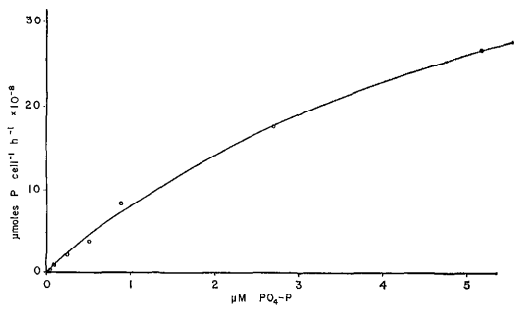

Fig. 6. Phosphorus uptake by *Peridinium*. The curve is the least-squares fit of a rectangular hyperbola to the data points. $k_s = 6.3$ µM P (SE = 0.9, df = 5); $u_m = 59.9 \times 10^{-8}$ µmoles cell^{-1} h^{-1} (SE = 5.6×10^{-8}).

a fraction of the maximum rate u_m, k_s is the half-saturation constant for uptake, and S is the average concentration of phosphate during the uptake interval. Because these rates were measured on washed cell suspensions incubated for an hour, they measure the capacity of the transport mechanism of the cells, rather than true uptake, i.e. surface adsorption, by the cells (Droop 1973). Moreover, the relation between the capacities measured in such washed cells and those of exponentially growing cells is uncertain. The Langmuir isotherms fit to the data (Eq. 1) are thus just empirical representations of many complex underlying physiological events. The very great difference between the behavior of *Peridinium* and *Dinobryon*, however (an order of magnitude difference in the k_s values) suggests that *Dinobryon* is in fact the more effective of the two at removing nutrients from dilute solutions.

Low half-saturation constants may well be characteristic of *Dinobryon* species in general, as *D. sociale* var. *americanum* also conforms to that pattern (Fig. 7). Two experiments are shown. Cells were suspended in phosphate-free media for different intervals before each, with the result that the more depleted cells gave greater uptake rates at any given concentration, although half-saturation constants were not

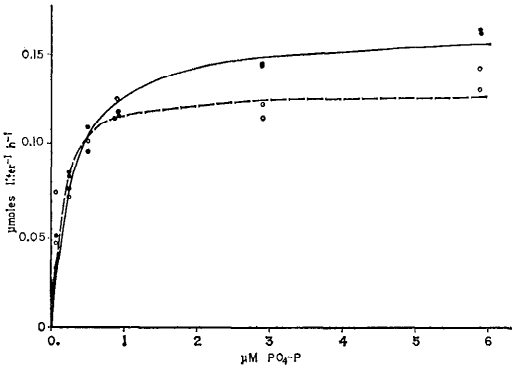

Fig. 8. In situ phosphate uptake in Mirror Lake, New Hampshire, during abundance of *Dinobryon divergens*. Upper curve (morning, 1100–1200 hours) $k_s = 0.27$ μM (SE = 0.04, df = 12), $u_m = 0.16$ μM h^{-1} (SE = 0.006); lower curve (afternoon, 1330–1430 hours) $k_s = 0.10$ μM (SE = 0.02, df = 11), $u_m = 0.13$ μM h^{-1} (SE = 0.006).

significantly affected ($p > 0.5$). Such behavior is harmonious with Rhee's (1973) discovery that internal stores of phosphorus inhibit phosphate uptake by *Scenedesmus* noncompetitively, affecting u_m but not k_s. That these low half-saturation constants are applicable to field populations is shown in Fig. 8, where uptake rates for phosphate are given for Mirror Lake, New Hampshire, at a time when the net plankton was dominated by *D. divergens*. I did not simultaneously sample the nannoplankton to evaluate the importance of the species to the total plankton. In this case, both u_m and k_s changed from morning to afternoon, but the factors underlying these changes were not investigated.

Temperature and ammonium dependences—Potassium and phosphorus provide a partial explanation for patterns of occurrence of *Dinobryon* in nature, but several other environmental factors can be shown to affect it as well. The seasonal distribution of *Dinobryon* in the pond is shown in Fig. 9, plotted with water temperature at 1 m. Thermal stratification was never strongly marked in the shallow pond. Cell densities ($\langle D \rangle$) are a lake average, derived as

$$\langle D \rangle = \sum_{i=1}^{3} D_i A_i / \sum_{i=1}^{3} A_i,$$

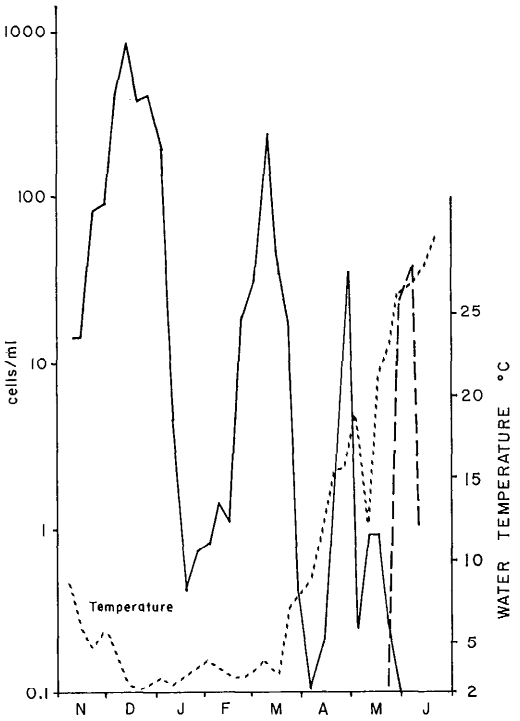

Fig. 9. Seasonal periodicity of *Dinobryon sertularia* (solid line) and *Dinobryon cylindricum* (dashed line) in the Boy Scout pond from November 1968 to June 1969, plotted with water temperature 1 m below the surface (dotted line).

where D_i is the measured cells $liter^{-1}$ at depth i and A_i is the cross-sectional area of the basin at that depth. *Dinobryon sertularia* maintained dominance only during periods when temperature was below 20°C. That the seasonal distribution has a physiological basis is evidenced by Fig. 10, where growth curves of axenic *D. sertularia* in artificial media show that the maximum temperature for growth lies between 16 and 20°C. *Dinobryon cylindricum* grows less well at 24° than at 16° or 20°, so the rising temperature of the water may have contributed to the decline of that species in early June.

Another chemical factor besides phosphorus which correlated inversely with the abundance of *Dinobryon* was NH_3-N, at concentrations of NO_3-N below 0.03 mg liter ^{1}. Ammonia declined from 1.0 to about 0.25 mg NH_3-N $liter^{-1}$ during the maximal

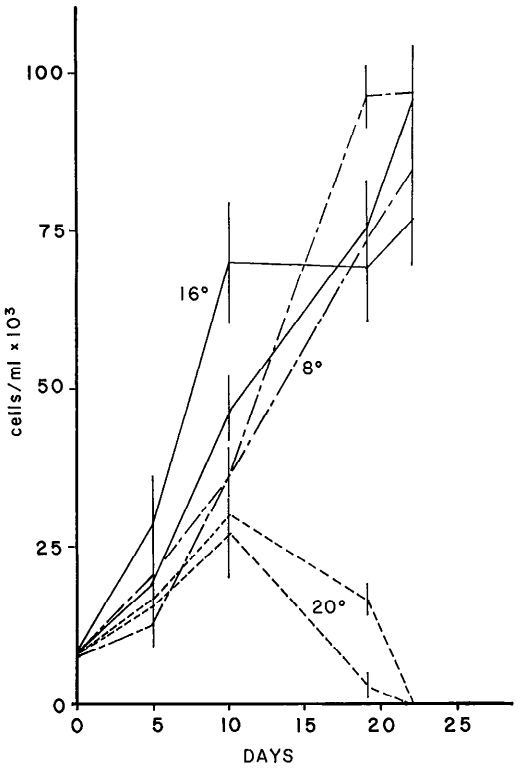

Fig. 10. Growth curves of individual culture tubes of *Dinobryon sertularia* grown at 8°, 16°, and 20°C. Plotted are means and 95% confidence intervals of cell counts.

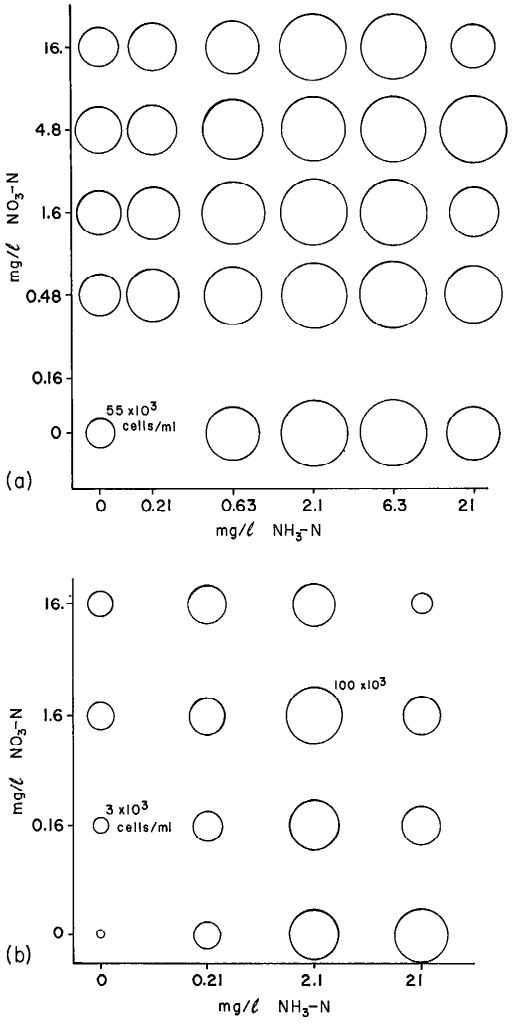

Fig. 11. Yields of *Dinobryon sertularia* (a) and *Dinobryon cylindricum* (b) at various combinations of NH_3-N and NO_3-N in DyIII medium. Areas of the circles are proportional to cell numbers.

expansion of the *Dinobryon* population (November–December: Fig. 9). The correlation with ammonium is apparently due not only to preferential utilization of nitrogen in that form, but also to the fact that growth per unit N is better when the element is supplied as NH_4 (Figs. 11a and b). I did these experiments using cells previously maintained in DyIII (Table 1), containing both nitrate and ammonium, thus precluding forced adaptation to a single nitrogen source. Because this apparent preference for ammonium can be observed with both axenic and bacterized cultures, and two species, it probably has validity for interpreting the field data, and it explains why ammonium levels reflect abundances of *Dinobryon* better than nitrate levels.

Vitamins and trace metals—Dinobryon sertularia requires biotin, thiamine, and B_{12} for sustained growth, determined from the relative growth of cells in media containing the vitamins singly or in various combinations after three serial transfers. No other organic substances are required, and the species is incapable of subsisting without light in the presence of sodium acetate, sodium lactate, or glycine. Several dozen other organic compounds, including auxins and vitamins, were added to light-grown cultures in attempts to stimulate growth

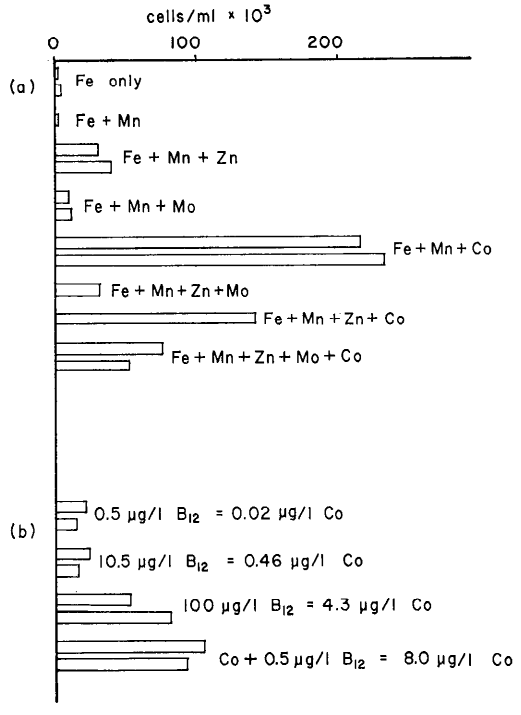

Fig. 12. a—Yields of *Dinobryon sertularia* in various mixtures of trace metals after three serial transfers. The trace metals were added at the concentrations shown in Table 1. b—Stimulatory effect of cobalt, added either as an inorganic salt or bound in vitamin B_{12}. When available, the yields of two replicate culture tubes are shown in both (a) and (b).

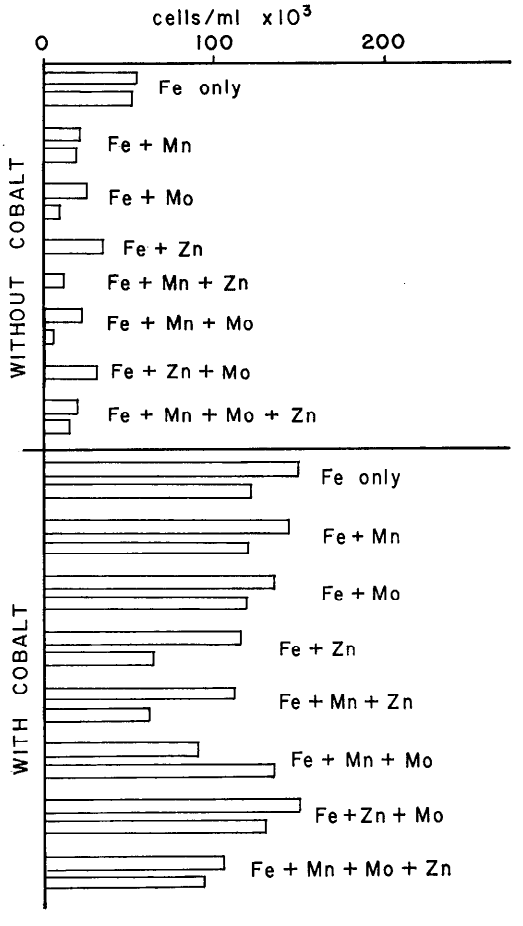

Fig. 13. Comparison of the yields obtained with and without addition of cobalt to cultures of *Dinobryon sertularia* in an experiment different from that shown in Fig. 12a. Plotted are the yields of replicate culture tubes after one serial transfer.

further, but without success. Such compounds as sodium glycerophosphate, adenylic acid, and uridylic acid can serve as the sole source of phosphorus for *D. sertularia*, however, so the cells may have a limited capacity for uptake of organics.

Substances other than vitamins added in trace amounts can stimulate growth. The response of *D. sertularia* to trace metals was studied in DyIII prepared with EDTA but without the metals, with trace metals then added to experimental tubes either singly or in combination at the concentrations normally used. An absolute requirement for iron was readily demonstrated; without iron growth virtually ceased after only one serial transfer. Even after three serial transfers some growth still occurred in tubes supplied with iron only (Fig. 12a), due presumably to trace metals added as contaminants with the nutrient salts of the medium and particularly with the buffer. The experiment showed nonetheless that various trace metal combinations stimulate growth (Fig. 12a). The most impressive results occur when cobalt is present in the mixture.

The presence of EDTA in the medium complicates interpretation of trace metal data because it chelates metals differentially. Consequently, to help explain whether the observed enhancement of growth was due to cobalt per se or to sub-

tle changes in EDTA binding affinities, increasing concentrations of cobalt were supplied in bound form as vitamin B_{12} (Fig. 12b). Because the cells responded similarly to free Co and to cobalamine-Co, the enhancement is probably due to a genuine cobalt requirement, rather than to chelation phenomena. In a separate experimental series, the effect of cobalt was notable even after one serial transfer (Fig. 13).

Discussion

The experiments show that *D. cylindricum*, *D. sertularia*, and *D. sociale* var. *americanum* are not inhibited by concentrations of phosphate much higher than any they commonly encounter in the field. A similar tolerance is exhibited by at least some strains of *D. divergens* (Munch 1972; Lehman personal observations). Presence of these organisms in water depleted of phosphate is due not to the toxic effect of phosphorus, but to the ability of *Dinobryon* species to extract that element from dilute concentrations at near-maximal rates. Misleading evidence for phosphorus toxicity nonetheless may appear when cultures or lake water are enriched with phosphorus as its potassium salt. Munch (1972), for instance, observed that filtered lake water enriched with 60 μg liter^{-1} P as sodium glycerophosphate or an artificial medium with even higher levels would favor growth of *D. divergens* and *D. cylindricum*, provided potassium concentrations were kept low. Inhibition of *D. divergens* appeared at 3.3–4.0 mg liter^{-1} K, but *D. cylindricum* was inhibited only at considerably higher levels, a pattern that agrees with the results of this study. These data, however, cannot serve to refute Rodhe's (1948) observations of phosphorus toxicity produced by enriching Lake Erken water with K_2HPO_4, since he added only microgram quantities of potassium. The disparate results could conceivably be due to ecotypic variation between strains. However, potassium toxicity has been shown to be moderated by calcium and magnesium ions, and other compounds may also be important, so that environmental fluctuations of the order of 1 mg liter^{-1} K might prove critical in some situations. Furthermore, my results are in terms of total yield in cultures, whereas effects on growth rates might be seen at considerably lower levels. Concentrations of potassium necessary to kill all cells of *Dinobryon*, and thus reduce yield to zero, might be much higher than those that could slow division rates to a point where population growth no longer exceeds sinkage and grazing losses in nature.

The data presented here suggest that inhibition by potassium limits at least the geographical distributions of several species, and possibly their seasonal distributions as well. In the Swedish Lake Ösbysjön, for example, *D. sertularia* appears in June a few weeks after the ice breaks and persists in the cool water until early autumn (Willén 1961). Its period of abundance matches exactly a summertime decrease in potassium, caused partly by precipitation of that element by clay colloids and partly by its uptake by littoral vegetation (Ahl 1966). The disappearance of *Dinobryon* corresponds to increased concentrations of potassium, as that element is released from dying vegetation. The data from the field are only circumstantial but nonetheless suggestive in light of culture studies.

Another observation which can be better understood in light of these experiments is the apparent instance of Mo deficiency in *D. sertularia*, which grew markedly when water of Castle Lake was enriched with the micronutrient (Goldman 1972). The biochemical basis for the limitation probably lies in the fact that nitrate reductase, necessary for utilization of NO_3, is a Mo-containing flavoprotein. Hewitt (1959) noted that in *Scenedesmus* a Mo requirement can be readily established on a nitrate medium but cannot be shown on media containing reduced forms of nitrogen, urea, or ammonium. Laboratory experiments with pure cultures of *D. sertularia* fail to demonstrate Mo deficiency when ammonium is present in the growth medium (Figs. 12a, 13), probably due to marked preferential utilization of NH_4-N

by the cells. In oligotrophic Castle Lake, however, where the cells are forced by circumstance to utilize nitrate—the source in greatest abundance—they presumably profit from an ample supply of Mo.

The very marked stimulation of growth produced by adding cobalt to the culture medium and the similar responses to its addition either as the metal alone or as Cobalamine-Co may mean that the cells of *D. sertularia* have a limited ability to manufacture vitamin B_{12}, which is improved by increased concentrations of cobalt, or that cobalt is required in quantity as a cofactor for other purposes. Whichever is the case, the magnitude of the response to the addition of cobalt to the growth medium suggests that environmental variations in availability of trace metals might alter species composition in nature, as Patrick et al. (1969) and Goldman (1972) have suggested.

Dinobryon sertularia uses phosphorus not only as inorganic phosphate, but also as organically bound phosphate in moderate-sized molecules: as glycerophosphoric acid (MW 172), uridylic acid (MW 324), and adenylic acid (MW 347). It can obtain nitrogen either from inorganic sources or from such organics as urea, glycine, adenylic acid, and uridylic acid. Its capacity to utilize these organic sources and Pascher's (1943) report of phagotrophism imply that *Dinobryon* is suited to occupy the water column when death and decline of previous bloom organisms release products of cell breakdown to solution. It is also at these times, when ambient levels of dissolved inorganic nutrients have become somewhat depleted, that the potentials of the cells for effective uptake of nutrients show their advantage.

Evidence that *Dinobryon* possesses efficient mechanisms for uptake of nitrogen and phosphorus is found in the experiments of Schelske and Stoermer (1972). They showed that populations of *D. divergens* could expand at the low levels of N and P found in Lake Michigan during July (e.g. <2 μg liter^{-1} PO_4-P) while the many diatom species present required simultaneous addition of N and P to the same water to increase their population sizes similarly. Low half-saturation constants for uptake of phosphate by *Dinobryon* species are implied by measurements made both in laboratory cultures and in lakes in my study.

Efficiency of nutrient uptake explains the oft-noted inverse correlations between numbers of *Dinobryon* and concentrations of phosphorus. When vernal blooms of diatoms or other phytoplankters reduce phosphorus to levels that limit their own growth, *Dinobryon* species compete effectively for what remains and may eventually dominate the plankton during periods of nutrient depletion. Phosphorus alone is not toxic, however, and the species can grow even at high concentrations of the element.

We must then ask why *Dinobryon* is usually outcompeted by other species when nutrients are abundant. Empirical studies by Eppley et al. (1969) with nitrogen, NH_4 and NO_3 and by Fuhs et al. (1972) with phosphate show that algae efficient at uptake of nutrients usually have lower maximum intrinsic growth rates than less efficient species. This seems to hold true even between strains of a single species (Guillard et al. 1973), an observation for which Doyle (1975) has proposed a theoretical rationale. These differences seem to represent two variations of an adaptive scheme for nutrient utilization. Some species exploit a resource-laden environment rapidly while others display measured efficiency in utilizing a limited resource. Energy tradeoffs among competing intracellular processes might preclude organisms from excelling at both simultaneously. *Dinobryon* probably does not appear during the vernal excess of nutrients in some lakes because it cannot keep pace with other more rapidly growing species. When these bloom organisms reduce nutrient concentrations to low levels, however, and their growth rates decline to a point that is balanced or exceeded by loss rates, the more efficient *Dinobryon* species hold a competitive advantage and their populations expand rela-

tive to others. The period of *Dinobryon* abundance is only transitory, however, as populations can be slowed or halted by such environmental factors as temperature, potassium concentration, or continued nutrient depletion. Grazing pressure from filter-feeding zooplankton may also be important: Tappa (1965), for example, reports that *Dinobryon* may constitute 95% of the gut contents of some *Daphnia* species when the chrysophyte is abundant.

Population dynamics of the *Dinobryon* species followed in this study appeared to be substantially dependent on the physicochemical environment. Potassium concentrations in the pond (Table 2) were below toxic levels, but temperature almost assuredly limited the seasonal periodicity of *D. sertularia*. Population expansions were correlated with declining concentrations of molybdate-reactive phosphate and ammonium nitrogen, at least some of which were due to nutrient uptake by the growing populations. The decline in May of *D. sertularia* was probably due to rising water temperature. Grazing may have caused the prior population crashes as no other, more likely, explanation emerged; but zooplankton counts available for the first 4 months of the study, including the period of maximum abundance of *Dinobryon*, fail to show any clear relation between chrysophyte numbers and population densities of Cladocera and copepods that could be considered evidence of grazing. Only the small rotifer *Filinia* was related at all to numbers of *Dinobryon*, and it showed a positive correlation based on the weekly samples. Its small size makes it an unlikely candidate as the grazer of the chrysophyte, however. Although grazing is not a satisfactory explanation for at least one of the *Dinobryon* declines, it remains a possible explanation for population declines during later months.

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