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Predominance of picoplankton and nanoplankton in eutrophic Calder Lake

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Abstract

A study was conducted to examine factors regulating the biomass of algal picoplankton in Calder Lake, a small eutrophic lake in southern New York state. A particular focus was a current paradigm which suggests that larger cells may dominate in nutrient-rich waters, while smaller cells may predominate only in oligotrophic waters. Over two years, phytoplankton biomass consisted predominantly (74% on average) of very small organisms; nanoplankton (<20 to 2 μm: 39%) and picoplankton (<2 μm to 0.2 μm: 35%), despite the presence of surface blooms of colonial cyanobacteria (*Microcystis aeruginosa*, *Anabaena limnetica*), and dense metalimnetic populations of the dinoflagellate *Ceratium hirundinella*. This dimictic system is characterized by relatively high levels of total P (max = 85, $\bar{x}$ = 9.7 μg P/L), inorganic P (max = 26, $\bar{x}$ = 4.5 μg P/L), and total inorganic N (max = 285, $\bar{x}$ = 85 μg P/L), but larger forms were rarely the most abundant. Unlike some marine systems, greater abundance of algal picoplankton was not associated with deeper strata (low light), or warmer temperatures. Data suggest that midsummer nutrient limitation, especially P-limitation, favors the development of pico- and nanoplankton in the limnetic zone of eutrophic lakes.

Introduction

The demonstration that bacterial-sized phytoplankton may constitute the majority of oceanic phytoplankton biomass and primary production is now well established (Waterbury *et al.*, 1986; Stockner, 1988). These microorganisms, termed picoplankton, include autotrophic cells from 0.2 to 2.0 μm in size (Sieburth *et al.*, 1978). Algal picoplankton may also represent a significant proportion of algal biomass in certain North American Great Lakes (Caron *et al.*, 1985; Fahnenstiel *et al.*, 1986) and in Lake Zurich (Chang, 1980). Studies on smaller oligotrophic and meromictic lakes also indicate the probable general importance of picoplankton in freshwaters (Paerl, 1977; Craig, 1984). Primary production is also thought to be dominated by freshwater picoplankters (Paerl & Mackenzie, 1977; Ellis & Stanford, 1982), although not all studies distinguish nano- from picoplankton. More basic information is still needed.

Studies on freshwater systems, while lagging behind the progress made in marine systems, have begun to recognize the implications of these findings within the context of lake dynamics. One study has recently addressed the basic question of time/depth/temperature relations. In Lake Ontario, picoplankton abundance is correlated with warmer temperature (Caron *et al*, 1985). Relative

and absolute abundance was greatest in August and much lower in April. No published studies are known which have examined freshwater picoplankton biomass in lakes throughout an entire year, when temperature and nutrient differences may be expected to influence their abundance. It has been proposed, particularly in marine systems, that oligotrophic waters (e.g. open ocean) may be expected to support relatively large picoplankton densities (i.e. relative proportion), while more nutrient-rich systems (e.g. coastal), are dominated by larger forms (Stockner & Antia, 1986). Some data suggest that picoplankton are also less common in eutrophic lakes (Paerl, 1977; Prepas *et al.*, 1988), and that smaller cells lose their competitive advantage at higher P concentrations (Lean & White, 1983). Another well-documented pattern in marine systems is the greater relative abundance and proportion of primary production by picoplankton in deeper waters (Li, 1986; Waterbury *et al.*, 1986) presumably due to greater photosynthetic efficiency of some strains of *Synechococcus* (Glover, 1985) in these assemblages. All these patterns deserve further examination in lakes. These ideas were tested in Calder lake over a two-year period.

Five basic questions were asked:

a) Is algal picoplankton biomass a major component of the total phytoplankton biomass in this eutrophic lake?
b) Is there any variability in picoplankton biomass over time?
c) Is there greater absolute or relative abundance of picoplankton in deeper waters?
d) Are picoplankton relatively more abundant during warmer months?
e) Are picoplankton relatively more abundant during periods of nutrient limitation?

Methods and materials

Study site and sampling

Phytoplankton were collected every 2–3 weeks, at 7 depths from Calder Lake, which is located at the biological station of Fordham University (Fig. 1).

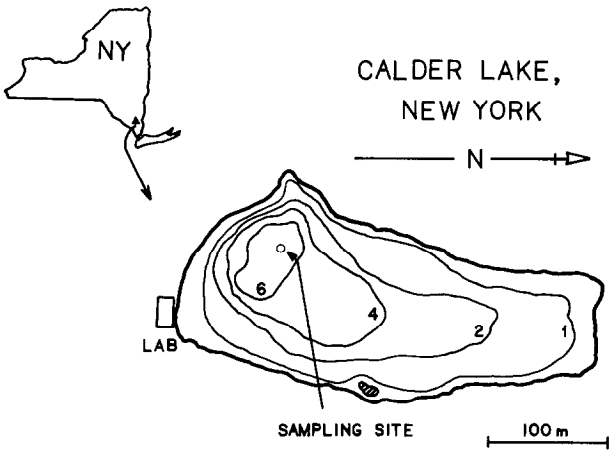

Fig. 1. Location of Calder Lake, indicating depth profile (m), sampling site and laboratory.

In Jan.–Mar. 1987, ice was thick enough to sample the lake, but in the same period in 1988 it was not safe to sample until ice breakup. Samples were analyzed in the limnology laboratory, located on the southern shore of Calder Lake. Calder Lake (3.9 ha) is a shallow ($\overline{Z}$ = 2.8 m), dimictic, eutrophic lake located in southern New York state. The lake is spring-fed and located within an undisturbed deciduous forest. The sampling station was located in the deepest location (Zmax = 6.7 m) in the lake. Chemical features of this system are presented in Table 1. The study has run from Jan. 1987 through Nov. 1988.

Table 1. Chemical characteristics of Calder Lake, NY (1987-1988; n = 154; data from seven depths, described in Methods).

Variable	Min.	Max.
pH	6.9	8.7
Total alkalinity (meq/L)	0.4	2.2
Na (mg/L)	5.0	6.0
Mg (mg/L)	5.9	7.1
Ca (mg/L)	9.4	16.6
nitrite (μg N/L)	<0.6	3.0
nitrate (μg N/L)	0.7	74
ammonium (μg N/L)	5.2	208
total N (μg N/L)	130	1080
reactive P (μg/L)	<1.0	26
total P (μg/L)	3.7	85
SiO_2 (mg Si/L)	0.1	1.6

Phytoplankton were divided into three size classes (Sieburth *et al.*, 1978): microplankton $<200\ \mu m$ to $>20\ \mu m$; nanoplankton $<20\ \mu m$ to $>2\ \mu m$; picoplankton $<2\ \mu m$ to $>0.2\ \mu m$. Lakewater was collected using a Masterflex (Cole-Palmer Co.) peristaltic pump, fitted with silicone tubing, at seven, 1 m intervals from 0.1, and 1 to 6 m depths. Microplankton were prefiltered *in situ* using (25 mm) in-line (nylon) filter units, fitted with 20 μm Nitex disks. At low pumping rates (<500 mL/min) very little pressure is exerted on the cells collected. Remaining fractions were separated in the laboratory (<30 min later). Water was collected for nutrient analyses at the same depths. Temperature profiles were measured using a YSI (model 57) temp/DO meter, underwater light using a Li-Cor PAR meter (model 185A) with submersible probes, and Secchi disk depth was recorded.

Laboratory procedures

Filtrate (-microplankton) from the plankton collections was filtered twice more in the laboratory using Nuclepore filters under low vacuum pressure (<25 to 100 mm Hg; Li, 1986). A series of seven 1000 mL Nalgene polysulfone filtration units were linked in series to filter all strata simultaneously. Nanoplankton were collected on 2.0 μm poresize filters (ca. 0.3–1.0 L/sample); picoplankton passed through and were later collected on 0.2 μm poresize filters (ca. 0.1–0.5 L/sample). Filters were placed in extraction vials containing 3–4 mL of neutral (w/$MgCO_3$) 90% acetone (A.P.H.A., 1985), ground using a glass pestle and extracted cold (4 °C) in the dark for 18 h. Extracts were made to 5 mL, centrifuged and measured for chlorophyll *a* (Chl *a*) concentrations spectro-photometrically, corrected for pheophytin *a* (Lorenzen, 1967). Water chemistry samples (excepting pH and total alkalinity) were filtered with 0.45 μm poresize filters before analysis. Soluble reactive P (SRP) was analyzed via the antimony-molybdate method; the same procedure was followed for total P, following a persulfate-H_2SO_4 digestion (Eisenreich *et al.*, 1975). NH_4-N was determined via phenol-hypochlorite and NO_3 was determined using the sulfanilamide/NNED, following Cd reduction to NO_2 (Mackereth *et al.*, 1978). Alkalinity was measured directly by titration with 0.02N H_2SO_4 (A.P.H.A., 1985). Na, Mg and Ca were analyzed by atomic absorption spectroscopy (Perkin Elmer 1100B).

Routine counts and identification of larger phytoplankton species were carried out by the inverted microscope method (Utermöhl, 1958). Many, but not all, picoplankton cells could be observed by this procedure, after cells had settled for >12 h (10 mL settling chambers). Numbers were verified by epifluorescence microscopy (Waterbury *et al.*, 1986). Cells were filtered onto pre-stained black (0.2 μm pore size) Nuclepore filters, and counted using a Zeiss Universal phase microscope (Hobbie *et al.*, 1977). Biomass is presented as Chl *a*, but a reasonable correspondence was found between picoplankton numbers and Chl *a* biomass. Data were compiled and analyzed using the Systat statistical program (Wilkinson, 1987).

Results

Proportion of total biomass

Over two years, picoplankton ranged between 1.7 and 67% of the total phytoplankton biomass (mean = 34.5%; $n = 161$), as chlorophyll *a*. In Calder Lake, picoplankton were typically the most abundant fraction in 1987 (significantly largest proportion; 0.41: Fig. 2), although nanoplankton was most abundant in 1988. For the two-year period 1987–88, pico- and nanoplankton on average, collectively made up ca. 74% of the total phytoplankton biomass. Despite the occurrence of dense deepwater populations of *Ceratium hirundinella* and surface blooms of the cyanobacteria *Microcystis aeruginosa* and *Anabaena limnetica*, smaller organisms were significantly more abundant and had significantly greater biomass (3-way pairwise ANOVA; for all possible date-depth comparisons).

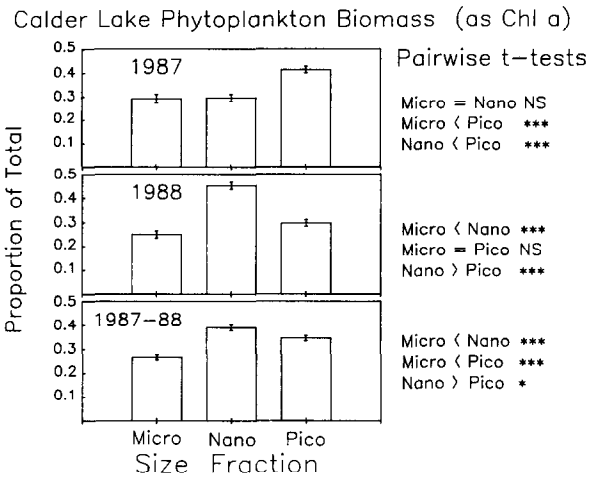

Fig. 2. Proportions of Calder Lake phytoplankton biomass (chlorophyll *a*) represented in three size classes, for 1987, 1988 and 1987–88. Pairwise t-tests based on all date-depth comparisons (NS = non-significant. * = $p < 0.05$, *** = $p < 0.001$; error bars = ± 1 SE).

Table 2. Summary of average phytoplankton biomass and its seasonal variation (CV in %) in three size fractions within the epilimnion (0.1 to 3.0 m) and hypolimnion (4.0 to 6.0 m) in Calder Lake, 1987–88 (biomass: μg/L Chl *a*; epilimnion $n = 91$; hypolimnion $n = 63$).

Stratum	MICRO		NANO		PICO	
	$\overline{X}$	CV	$\overline{X}$	CV	$\overline{X}$	CV
Epilimnion	0.47	70%	0.78	104%	0.54	50%
Hypolimnion	1.41	275%	2.11	196%	1.62	177%

Variability over time

Absolute biomass levels in all three size classes varied seasonally, although in surface (1 m) waters, micro- and nanoplankton biomass levels were typically more erratic seasonally (Fig. 3). This is also apparent from the coefficients of variation in each (CV_{micro} = 72%, CV_{nano} = 86%, CV_{pico} = 56%). Microplankters, chiefly colonial cyanobacteria (*Microcystis aeruginosa*, *Anabaena limnetica*) and large dinoflagellates (*Ceratium hirundinella*) had marked peaks in summer and autumn months, while nanoplankton pulses (*Scenedesmus* sp., *Cryptomonas* spp.) were rather unpredictable. There were generally higher numbers of picoplankton (cells and Chl *a* biomass) in the summer, although no dramatic pulses were observed. Seasonal biomass patterns in deeper water (5 m) are more varied (note also different scale), and total biomass reached greater densities in all three fractions (Fig. 4). However, picoplankton and nanoplankton (CV = 65%, 74%, respectively) were much less variable than micro-

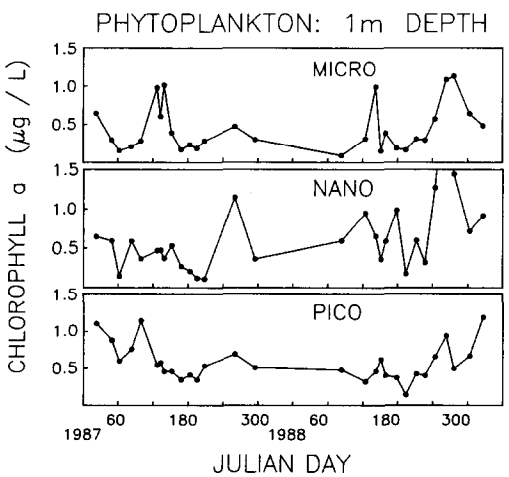

Fig. 3. Temporal variations in Calder Lake phytoplankton biomass at 1 m, represented in three size fractions, from 1987–1988 (point exceeding range in nano plot = 2.9 μg Chl *a*/L).

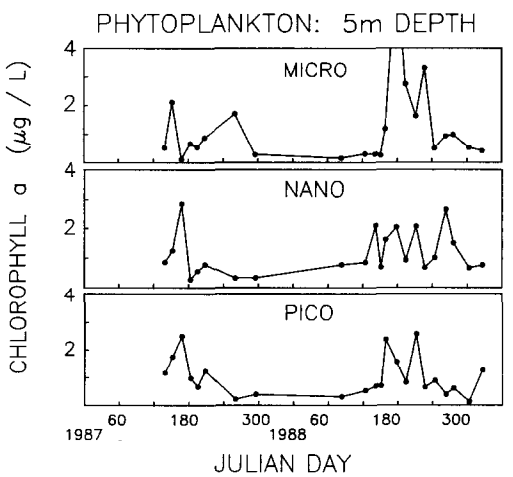

Fig. 4. Temporal variations in Calder Lake phytoplankton biomass at 5 m represented in three size fractions, from 1987–1998 (note different scale from Fig. 3; point exceeding range in micro plot = 6.9 μg Chl *a*/L).

plankton (CV = 123%), seasonally. Unlike surface assemblages, the wax and wane of each size class at 5 m varied similarly, with very low levels during the early spring and winter. Seasonal variation in picoplankton biomass within the epilimnion (0 to 3 m) and hypolimnion (4 to 6 m) was considerably less than that observed in larger size classes (Table 2). This relative constancy in picoplankton occurred within a highly variable chemical and physical regime (see later section), such as summer stratification and winter ice cover.

Greater abundance with depth

The abundance of picoplankton and larger size classes varied with depth, but patterns changed seasonally and year to year (Fig. 5). For example, on 19 May 1987, picoplankton biomass varied markedly with depth and was greatest at the metalimnion. By 3 July, biomass of all size fractions was relatively uniform versus depth, even though stratification had set in with a vertical temperature gradient of 10.4 °C. Picoplankton did, however, represent the largest proportion of phytoplankton biomass throughout the water column. The 1988 patterns for nearly identical dates were quite different. In May, pico densities did not vary with depth, yet nano biomass varied 4-fold (a metalimnetic maxima of *Cryptomonas erosa*, small *Synura* colonies, and other flagellates). By July, microplankters (*Ceratium hirundinella*) were the most heterogeneous vertically, with the smaller fractions less so. Picoplankton biomass was roughly 29 times greater, and nanoplankton 4.7 times greater near the lake bottom, than at the surface. However, microplankton increased by 130-fold with depth. Nearly unialgal communities of *Ceratium* reached a deepwater maximum (at 6 m) of 30.5 μg Chl *a*/L.

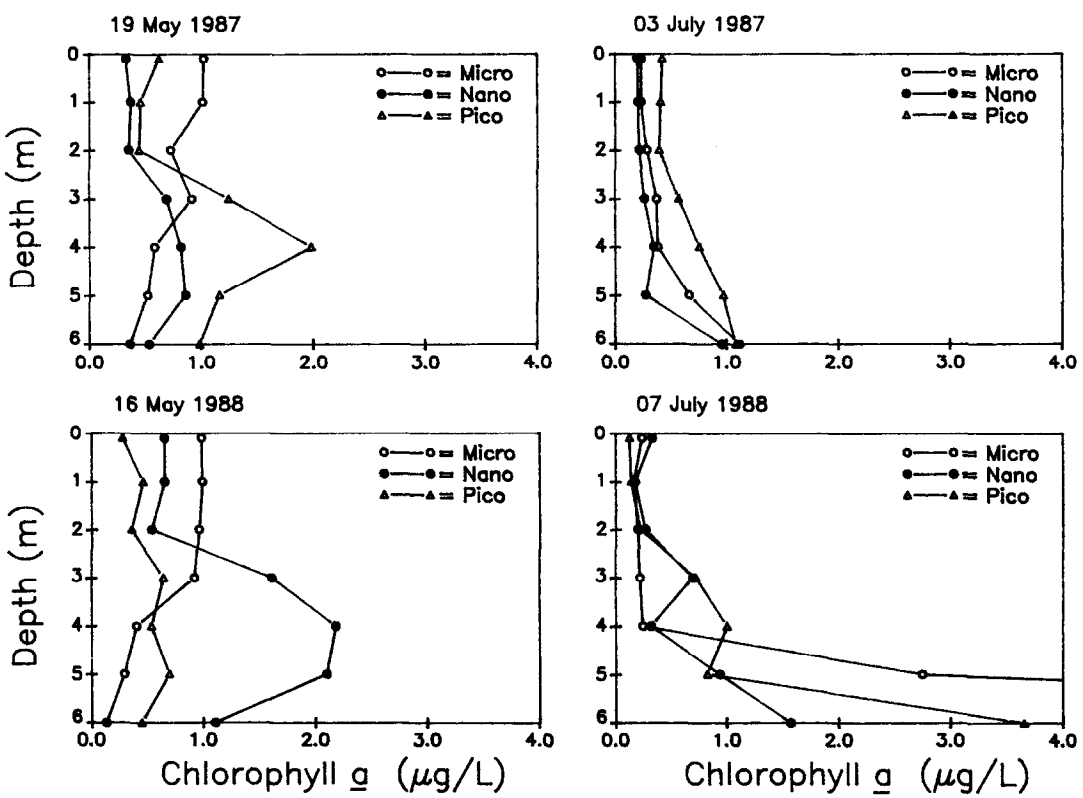

Fig. 5. Vertical and temporal variations in phytoplankton biomass in Calder Lake, NY, separated into three size classes. Dates of sampling in two seasons are compared with analogous data from two years (1987–88; value for micro at 6 m depth on 07 July 1988 = 30.5 μg Chl *a*/L).

The above data suggest that distinct vertical patterns in picoplankton may exist. A regression between picoplankton biomass (Chl *a*) and depth for the two-year dataset was very highly significant ($Y = 0.299 + 0.107$; $P < 0.001$). However, nanoplankton ($P < 0.001$, slope = 0.378) and microplankton ($P < 0.05$, slope = 0.248) were also significantly correlated. Thus, a second regression between the proportion of total biomass as picoplankton, with greater depth was non-significant ($P > 0.4$), indicating that picoplankton were not relatively more abundant than larger size fractions at greater depth.

Greater abundance in warmer temperatures

Epilimnion temperatures in Calder Lake during 1987–88 ranged from 0.5 to 28.7 °C, and varied as much as 12 °C with depth during summer stratification. Data (Fig. 5) indicate that phytoplankton biomass was perhaps greater during the summer, but an overall regression found that picoplankton biomass (both absolute and relative) was not strongly related to temperature ([Chl *a*]: $P > 0.5$; proportion of total: $P > 0.1$). Apparently, there is a temperature optimum near 17 °C, but abundance declines above that.

Greater abundance during nutrient limitation

Calder Lake was characterized by marked seasonal variation in key nutrients, as indicated by data for the 1 m stratum (Fig. 6). N and P became depleted during summer months, with epilimnion NO_3-N and NH_4^+-N averaging 7.8 and 49.3 μg N/L, respectively, and SRP averaging 1.5 μg P/L in 1988. Following summer 1988, there was apparently little regeneration of either nitrate or SRP to the epilimnion following autumn turnover (day 270). However, NH_4^+-N concentrations did

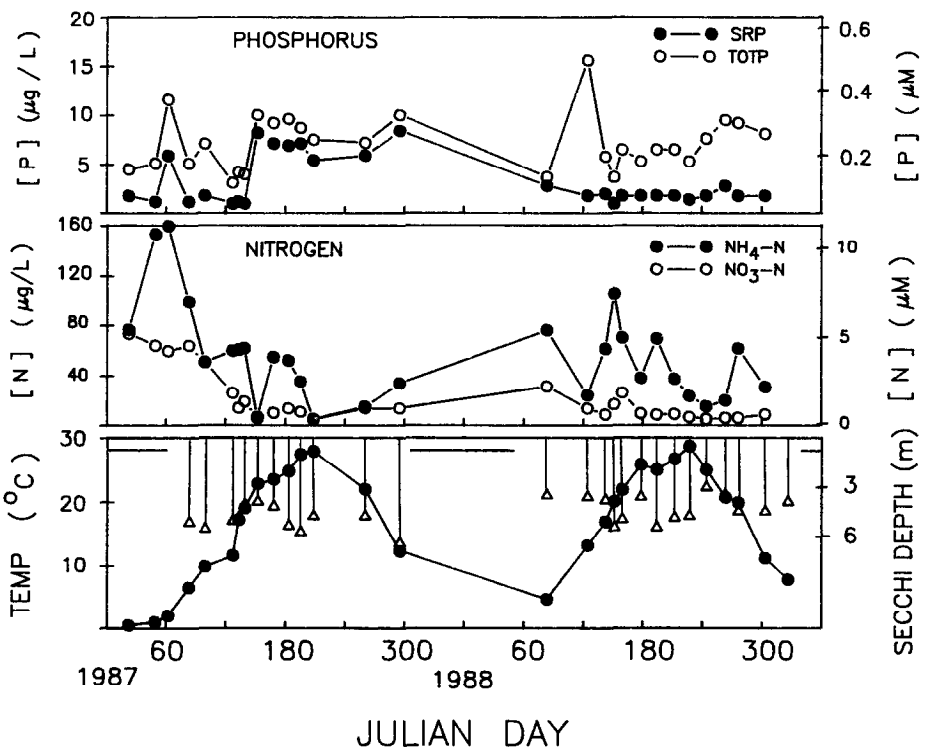

Fig. 6. Temporal variation in phosphorus (SRP = soluble reactive – P, TOTP = total soluble P), nitrogen, temperature (all at 1 m depth) and Secchi depth (open triangles) within Calder Lake, 1987–1988. Horizontal bars in the temperature plot indicates ice cover.

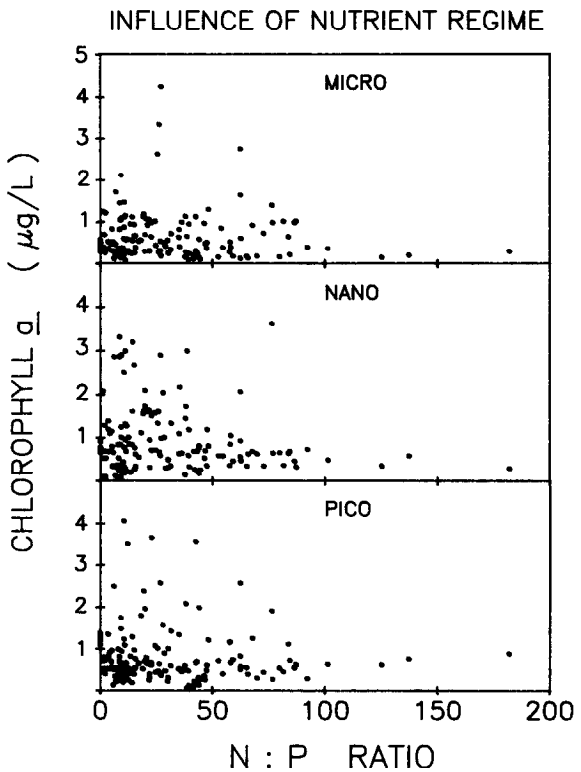

Fig. 7. Relation between N:P (atomic) ratios in lakewater and phytoplankton biomass in Calder Lake, separated into three size fractions (defined earlier).

increase in late 1988. The second year was warmer than 1987, which was reflected in the roughly ten-fold greater phytoplankton biomass during midsummer (1987 mean = 2.1; max = 9.2 μg Chl *a*/L; 1988 mean = 3.8; max = 50.2 μg Chl *a*/L). N:P (atomic) ratios in Calder lakewater varied from 1.2 to 182. This broad and dynamic range of chemical conditions was compared against phytoplankton biomass for each size fraction (Fig. 7).

While some general negative relation was observed, no statistically significant relation was uncovered (first and second other polynomials tested). Regressions between SRP and phytoplankton biomass were also non-significant for all three size fractions. Another means to examine this is by running pairwise t-tests to compare biomass levels of the three size fractions at the extremes of N:P ratios. Ratios >30 were defined as P-limited, while those <15 were judged to be N-limited (after Rhee & Gotham, 1980). Results indicate that under both extremes, smaller size fractions constituted a larger proportion of phytoplankton biomass (Table 3), yet nano- and picoplankton levels were not significantly different. Under intermediate nutrient levels, when neither N or P are probable single limiting nutrients, the smaller size fractions were no longer more abundant than microplankton.

Discussion

Picoplankton biomass in Calder Lake (max = 18, mean = 0.97 μg Chl *a*/L) exceeds the maximum biomass densities reported for some lakes (Stockner, 1988), although the development of very large populations have been observed in other eutrophic systems (Drews *et al.*, 1961). These data do not support the contention (Stockner & Antia, 1986; Prepas *et al.*, 1988) that picoplankton are less abundant in eutrophic lakes. The lack of marked seasonal shifts in pico-

Table 3. Pairwise t-tests of relative biomass levels in pico-, nano-, and microplankton in Calder Lake during P and N limitation. Data are from all possible date/depth combinations (n = 58 for P-limitation, n = 71 for N-limitation, n = 32 for intermediate limitation; p = probability, NS = nonsignificant, ** = $p < 0.01$, *** = $p < 0.001$).

Comparison	P-limited		N-limited		Intermediate	
	Greater fraction	P	Greater fraction	P	Greater fraction	P
Micro vs. Nano	Nano	***	Nano	***	NS	
Micro vs. Pico	Pico	**	Pico	***	NS	
Nano vs. Pico	NS		NS		Nano	**

plankton biomass in Calder Lake stands in contrast to well-established patterns in many marine communities, where biomass (judged from cell numbers) may shift more than two orders of magnitude over an annual cycle (Waterbury *et al.*, 1986). Picoplankton densities in experimentally P-fertilized Kennedy Lake also varied by more than two orders of magnitude (Stockner & Shortreed, 1988). However, in Calder Lake, the most abrupt and dramatic shifts were seen in microplankton biomass (esp. *Ceratium hirundinella*), with a 30-fold increase in 16 days. This species, when isolated from Calder Lake, apparently requires about 15 days under 'optimal' culture conditions to produce a 10-fold increase in cell numbers (Bruno & McLaughlin, 1977). Very warm temperatures in the lake in 1988 may have contributed to this.

Some vertical patterns in picoplankton biomass were identified. A significant positive regression indicated that on average, picoplankton biomass increased at a rate of 0.299 μg Chl *a*/L per meter of depth. Total phytoplankton biomass was also positively correlated. Hence, the relative biomass of picoplankters was not any greater. However, light levels in Calder Lake do attenuate markedly with depth (e.g., at 2 m, I = 5.4% of PAR measured at surface; 16 Sep 1988). It is thus concluded that algal picoplankton in Calder Lake, unlike that in some marine systems (e.g. Glover, 1985; Waterbury *et al.*, 1986), is not relatively more abundant in deeper water, where light may become limiting. Further study is still needed on the question of low light adaptation (Glover, 1985), especially in freshwater systems, where water clarity can fluctuate rapidly (Wetzel, 1983).

The lack of a statistical correlation between either absolute or relative biomass of picoplankton with temperature is not surprising. Temperature responses of microorganisms should be expected to exhibit some pattern of optima, but this may not result in a linear response over the entire range observed in Calder Lake. A more reasonable test, that of the proportion of the phytoplankton represented by the pico fraction, in fact shows no trends or optima. This is less easily explained. While size-based functional patterns in phytoplankton are useful for understanding growth rates, production and other processes it should be remembered that these represent a collection of multispecies assemblages, each of which may have different optima and limitations. However, this is in contrast to one other freshwater study (Caron *et al.*, 1985), in which picoplanktonic cyanobacteria abundance and relative abundance were positively related to temperature. The predominance of pico- and nanoplankton in Calder Lake is correlated strongly with periods of nutrient limitation. During N and P surplus, these smaller cells are not more abundant than microplankton. An artificial reduction in sediment P in Lake Trummen resulted in a shift from a 'net plankton' ($>20\,\mu$m) to a 'nanoplankton' ($<20\,\mu$m) community (Gelin & Ripl, 1978), although cells <20 and $<2\,\mu$m were not differentiated. By manipulating natural assemblages, Suttle & Harrison (1988) found that greater supply ratios of N : P (= P-limitation) result in the dominance of the picoplankter *Synechococcus* within an oligotrophic phytoplankton community. In general, much of the study of algal ecology has centered around the paradigm that phytoplankton are frequently nutrient limited (Hecky & Kilham, 1988). This is clearly the case for microplankton in Calder Lake, but this cannot be as easily generalized for smaller species. Nanoplankton and picoplankton did not decline in responde to the depletion of nutrients within the epilimnion during summer stratification. A lesser degree of seasonal and vertical variation in picoplankton also suggests that these organisms are perhaps less susceptible to nutrient limitation.

The predominant view that freshwater ecosystems are principally phosphorus limited is well founded (Schindler, 1977), but the specific response of picoplankton may be quite different from that of better-known, larger forms. These organisms may also have profound effects on nutrient cycles, perhaps via superior uptake and storage of these key elements (Lean, 1973; Currie & Kalff, 1984; Wehr *et al.*, 1987). An abundant literature from laboratory studies indicate greater nutrient uptake rates and lower minimum require-

ments among smaller cells in culture (Glover, 1985). At present, however, the relative importance of various phytoplankton size classes as sinks or sources of N or P, and their role in nutrient fluxes within lakes is not well known. Perhaps picoplankters are distinct from larger phytoplankters in being relatively immune from temporal nutrient limitation, which has been thought to regulate the wax and wane of phytoplankton communities.

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